University of Alberta

THE ASSESSMENT OF LIMNOLOGICAL CHANGES USING SEDIMENT VISIBLE-NEAR-INFRARED (VNIR) SPECTRAL PROPERTIES

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

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Dedicated
To
My parents
And
My wife

ABSTRACT

Visible-near-infrared (VNIR) reflectance spectroscopy has made it possible to rapidly and non-destructively assess the chlorophyll content of plants and natural waters. However, to date this approach has not been applied to pigments preserved in lake sediments. The potential for using VNIR reflectance spectroscopy to assess chlorophyll *a* concentrations in lake sediments is explored in this thesis, with the ultimate goal of developing a new tool for reconstructing lake trophic histories. Reflectance spectra of lake sediments preserve salient troughs near 675 nm that covary in magnitude with concentrations of chlorophyll *a* and associated pheopigments. This feature is expressed in sediments from lakes with highly contrasted limnological regimes, confirming the ubiquity of this signature in any lake sediment that preserves chlorophyll and chlorophyll degradation products. Further critical assessments show that diagenetic degradation of chlorophyll *a* is a minor concern with respect to reconstructing past trophic histories from lake sediments using VNIR reflectance. This is primarily because red absorption near 675 nm incorporates both chlorophyll *a* and its degradation products. These various analyses demonstrate that VNIR reflectance spectroscopy may be used to infer chlorophyll in lake sediments, thus potentially providing a new methodology for reconstructing the evolution of lake trophic status.

PREFACE TO THE THESIS

The structure of my thesis is in paper format and is in four chapters. The first is a general introduction. The second and third are primary data chapters, and the fourth is a conclusion with implications for further study.

All co-authors participated and contributed in preparing each manuscript. Dr. Alexander P. Wolfe organized and prepared the final version of the first manuscript (Chapter 2). Below are cited the manuscripts as submitted and prepared for publication in the scientific journals. The citation and authorship of these manuscripts are as follow:

Chapter Two:

Das, B., Vinebrooke, R. D., Rivard, B., Sanchez-Azofeifa, A., and Wolfe, A. P. Inferring lake sediment chlorophyll concentrations with reflectance spectroscopy: a novel approach to reconstructing historical changes in the lake trophic status of mountain lakes. Reviewed and re-submitted in revised form to the *Canadian Journal of Fisheries and Aquatic Sciences* (September 2004).

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CHAPTER ONE: General Introduction

Nutrient enrichment in aquatic ecosystems, whether resulting from watershed disturbance, agricultural effluent, industrial development, or atmospheric deposition, continues to be a significant threat to global freshwater resources (UNEP-IETC, 1999). Nutrient enrichment, or eutrophication, impairs water quality and dramatically alters aquatic community composition (Schindler, 2000). Long-term monitoring data, which are rare or absent for most lakes, become essential in assessing the rate and magnitude of nutrient enrichment, thus providing a context for proper water quality management.

Paleolimnological approaches can provide long-term data sets, and have been used to examine a variety of environmental issues relating to freshwater eutrophication (Smol, 2002). Many paleolimnological indicators have been used to infer the legacy of nutrient enrichment from the sediment record, including siliceous microfossils (diatoms and chrysophytes), invertebrates, pigments, and geochemistry (Vinebrooke and Leavitt, 1999; Quinlan et al., 2002; Itoh et al., 2003; Saros et al., 2003; Wolfe et al., 2003).

Fossil pigments, in particular the chlorophylls, are preserved in lake sediments and provide excellent proxies for reconstructing trophic status (Vinebrooke et al., 1998; Leavitt and Hodgson, 2001). The chlorophylls, produced by all photosynthetic organisms, are the most abundant pigment type found in lake sediments. The amount of fossil chlorophyll preserved in sediments is determined by several factors (Hurley and Armstrong, 1990; Leavitt, 1993; Hodgson et al., 1998), including (1) original source and relative contributions from autochthonous and allochthonous sources; (2) rates of transport to sediments; and (3) post-depositional processes (diagenesis). A list of pigments found in lake sediments has been compiled in Table 1.1.

Although pigments have been shown to be well-preserved in a wide range of lacustrine sediments, considerable transformation of original compounds (or primary photosynthates) can occur owing to diagenesis (Hurley and Armstrong, 1990). In general, aerobic conditions accelerate post-depositional degradation, while anaerobic conditions enhance preservation. A pigment degradation and deposition model developed by Cuddington and Leavitt (1999) suggests that degradation due to herbivore grazing is the most dominant process in diagenesis of fossil pigments. The model also suggests that processes and regulation of pigment fluxes within the water column along with algal production regulate pigment sedimentation and preservation in lake sediments. A simple pathway of chlorophyll *a* degradation is shown in Figure 1.1. Typically, pheophytin *a* is considered a direct breakdown product of chlorophyll *a*, whereas pheophorbide *a* has been used as a proxy for herbivore grazing, given it's more complex pathway from chlorophyll *a* (Leavitt et al., 1994). However, it has also been shown that both pheophytin *a* and pheophorbide *a* can be produced solely from algal chlorophyll *a* degradation in the absence of grazing (Head et al., 1994). A range of different methods exists to determine pigment concentrations in lake sediments, and most of these involve wet chemistry and chromatography, and are thus inherently time-consuming and expensive. This thesis explores the potential of visible-near-infrared (VNIR) reflectance spectroscopy as a technique for determining chlorophyll *a* concentrations in lake sediments. The successful development of this technique, which is both rapid and non-destructive, potentially opens new horizons with respect to reconstructing lake trophic status from the lake sediment record.

A brief review of sediment pigment measurement techniques

Various spectrophotometric and spectrofluorometric techniques have been used to measure pigment concentrations in lake sediments over the last few decades. However, most of these methods have drawbacks including poor precision with respect to chlorophylls, non-specificity towards chlorophyll degradation products, and quantification problems associated with calibration equations (Hurley and Armstrong, 1990). For example, thin-layer chromatography (TLC) can be used to detect individual chlorophylls and partially track their transformation in sediments, yet it is largely qualitative, and unable to resolve either low chlorophyll concentrations or detect carotenoids. High performance liquid chromatography (HPLC) has become the most common technique in detection of individual pigments and their degradation products, because of greatly enhanced accuracy and precision. However, HPLC is time-consuming and expensive. Visible-near-infrared (VNIR) reflectance spectroscopy represents a potential new method for estimating chlorophyll in lake sediments. While reflectance spectroscopy has been used widely in limnology (Gitelson 1992, Mittenzway et al. 1992, Rundquist et al. 1996, Schalles et al. 1998, 2000) and paleolimnology (Nilsson et al. 1996, Korsman et al. 1999, Malley et al. 1999, Rosén et al. 2000) it has not yet been applied specifically to sedimentary chlorophylls in lake sediments, with the explicit objective of reconstructing lake trophic history.

Reflectance spectroscopy

Reflectance spectroscopy is a rapid, non-destructive analytical technique that identifies compounds by fingerprinting their light emission-absorption spectra (Clark,

Table 1.1: A list of the common pigments preserved in lake sediments and their associated precursor organisms (adapted from Rowan, 1989; Leavitt and Hodgson, 2001).

Pigments	Source organisms	Preservation in lake sediments
Chlorophyll *a*	All higher plants and all algae	Very common
Chlorophyll *b*	All higher plants, *Chlorophyceae*, *Euglenophyceae*	Moderately common
Pheophytin *a*	Derivative of chlorophyll *a*	Common
Pheophorbide *a*	Derivative of chlorophyll *a*	Common
β - carotene	All higher plants, all algae, some photoautotrophic bacteria	Common
Alloxanthin	*Cryptophyceae*	Moderately common
Fucoxanthin	*Dinophyceae*, *Bacillariophyceae*, *Chrysoyphyceae*	Moderately common
Diatoxanthin	*Bacillariophyceae*, *Dinophyceae*, *Chrysophyceae*	Moderately common
Lutein	*Chlorophyceae*, *Euglenophceae*, higher plants	Moderately common
Zeaxanthin	*Cyanophyceae*	Moderately common
Myxoxanthophyll	*Cyanophyceae*	Rare
Echinenone	*Cyanophyceae*	Rare
Isorenieratene	*Chlorobiaceae* (Photoautotrophic bacteria)	Rare

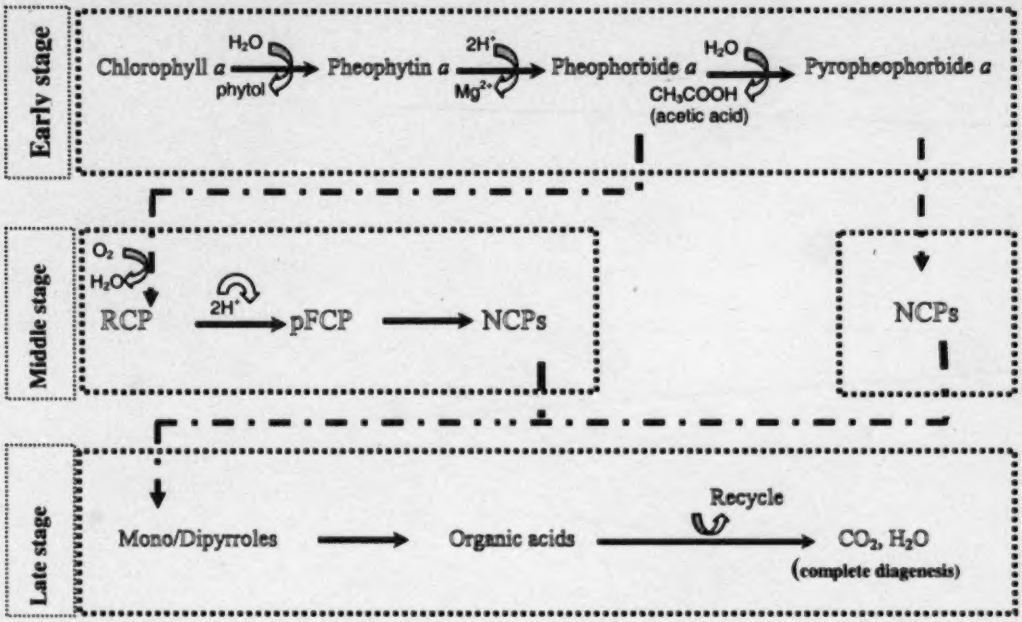

Figure 1.1: A simple flowchart illustrating the pathway of chlorophyll *a* degradation at different stages of diagenesis (adapted from Takamiya et al., 2000). Abbreviations: NCPs, nonfluorescent chlorophyll products; pFCP, primary fluorescent chlorophyll products; RCP, red chlorophyll products.

1999). When photons enter into a material (solid, liquid, or gas) some proportion is reflected from the sample surface (reflectance), some passes through it (transmittance), and some is absorbed (absorption). Reflectance spectroscopy is thus influenced by both the attenuation of a light beam as it passes through a sample, and reflection from the sample surface. These processes modulate the quantity of electromagnetic radiation, so that distinct wavelength signatures become detectable, arising from the different constituents of a particular substance. Visible-near-infrared spectroscopy is sensitive to many types of organic molecules and exploits absorbance within the VNIR region (400-2500 nm), which in turn are largely the results of molecular bending and stretching between light atoms (e.g., C-H, O-H, N-H, S-H, CH_2, C=O, COO-, C=C, etc.; Clark, 1999).

The VNIR spectral behavior of chlorophyll *a* in natural lake waters and controlled enclosures (mesocosms) has been studied in considerable detail, and several distinctive spectral features have been identified (Figure 1.2). Several reflectance peaks and troughs arise in the visible and nearest infrared portion of the spectrum, between 400 and 800 nm (Schalles et al., 1998; Schalles and Yacobi, 2000). These features include: (1) a trough around 430-450 nm that corresponds to the blue absorption band of chlorophyll *a*; (2) carotenoid absorption in the region 480-520 nm; (3) a green peak in reflectance centered on 600 nm and representing the minimum of total pigment absorption; (4) a major chlorophyll *a* absorption band near 670-675 nm; and (5) a reflectance peak near 750 nm which represents near-infrared scattering associated with low pigment absorption. A typical spectrum of pigment reflectance in water is reproduced in Figure 1.2. It is the combination of characteristic algal reflectance signatures such as these, and the

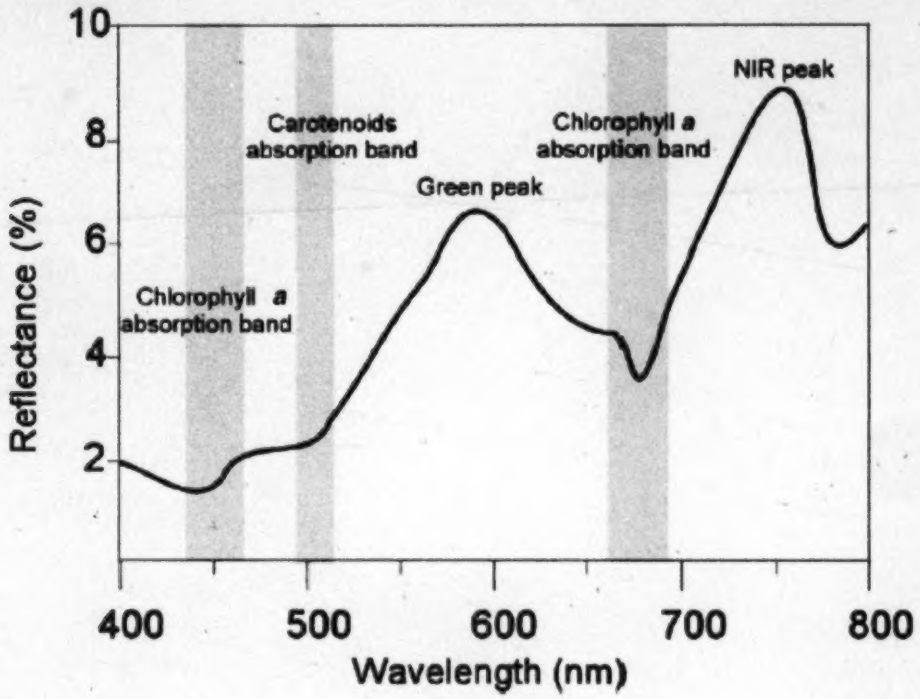

Figure 1.2: A typical visible reflectance spectrum of living diatom cells (*Synedra* sp.) in lake water medium (adapted from Schalles et al., 1998). (NIR = near-infrared)

realization that chlorophylls have preservation potential in lake sediments, that form the underlying research question addressed by this thesis: can VNIR reflectance spectra from lake sediments be used to detect fossil chlorophyll?

Rationale of the thesis and research hypotheses

It has been well established that chlorophyll *a* absorbs in the red-near-infrared (NIR) portion of the electromagnetic spectrum with a very high degree of sensitivity (Gitelson, 1992, Mittenzway et al., 1992, Quibell, 1992, Rundquist et al., 1996, Schalles et al., 1998, Méléder et al., 2003). Thus, these spectral characteristics have the potential to be applied to chlorophyll *a* disseminated as a trace organic constituent in lake sediments. If spectral signatures can be translated to sedimentary chlorophyll *a* concentrations, this information can be used in paleolimnological inferences of past lake trophic status.

The objectives of this thesis are to:

(1) assess the feasibility of applying VNIR reflectance spectroscopy to predict and reconstruct fossil chlorophyll *a* from lake sediment samples;

(2) evaluate the consequences of chlorophyll diagenesis on sediment VNIR spectra;

(3) develop inference models for predicting lake sediment chlorophyll concentrations from sediment VNIR spectra; and

(4) apply in a preliminary fashion, these models to reconstruct historical changes in sediment chlorophyll concentrations, and hence lake trophic status change.

Outline of the thesis

This thesis comprises four chapters, of which the second and third contain the primary data and are presented as separate, but related, manuscripts prepared for publication in the peer-reviewed literature. Background information, rationale, and specific objectives are summarized briefly here (Chapter 1), whereas general conclusions and suggestions for future research are proposed in Chapter 4. As far as the data chapters, Chapter 2 is essentially a feasibility study of the applicability of VNIR reflectance spectroscopy in paleolimnology, using a suite of alpine lake sediments from a well-studied region with respect to independent proxies (Wolfe et al., 2001, 2003). This pilot study, which has been reviewed and re-submitted in revised form to the *Canadian Journal of Fisheries and Aquatic Sciences*, suggests that chlorophyll *a* in lake sediments can indeed be remotely sensed with VNIR reflectance spectroscopy. In Chapter 3, this encouraging result prompted a more critical consideration of pigment degradation processes and their impacts of the sediment VNIR spectral properties. This chapter concentrates on the characterization of algal VNIR spectra in relation to photo-degradation and different redox conditions. The results of this study suggest that both primary and degraded chlorophyll *a* (*i.e.*, pheopigments) absorb in similar spectral regions, thus minimizing the potential impacts of diagenesis upon VNIR-based chlorophyll inferences. A manuscript destined for the *Journal of Paleolimnology* is in preparation from these results.

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CHAPTER TWO: Inferring lake sediment chlorophyll concentrations with reflectance spectroscopy: a novel approach to reconstructing historical changes in the trophic status of mountain lakes

INTRODUCTION

Among the most important applications of paleolimnology is the reconstruction of historical changes in lake trophic status, including those pertaining to cultural eutrophication (Hall and Smol 1999). To this end, robust methodologies have been developed using diatoms (Reavie et al. 1995; Bennion et al. 1996), sediment geochemistry (Engstrom et al. 1985; Schelske et al. 1986), and combined multi-proxy approaches (Fritz et al. 1993; Anderson and Rippey 1994). Sedimentary pigments (chlorophylls, carotenoids, and their derivatives) are of considerable utility in this objective, in part because they enable the quantification of past production by algal groups that do not produce morphological fossils (Millie et al. 1993; Leavitt and Hodgson 2001). However, the analysis of sedimentary pigments is expensive and labor-intensive, which ultimately limits the attainable spatial and temporal resolution of analyses. Because lake primary production is also linked to climate (Schindler et al. 1990; Hobbie et al. 1999; Flanagan et al. 2003), the development of techniques that facilitate the rapid assessment of lake trophic changes from the sediment record is increasingly of broad relevance.

Reflectance spectroscopy is a powerful tool for lake sediment analysis, in part because it is rapid, reagent-free, and non-destructive (Korsman et al. 1999, 2001). To date, applications include calibration of visible-near-infrared (VNIR; 400-2500 nm) spectra to air temperatures for paleoclimate reconstruction (Rosén et al. 2000), and use of

the NIR band (780-2500 nm) to infer organic and inorganic sediment constituents (Nilsson et al. 1996; Malley et al. 1999;). In paleoceanography, green reflectance (550 nm) has been used as a quantitative measure of sediment color (Peterson et al. 2000). However, these studies have not explored explicitly the relationships between localized spectral features and sediment geochemical composition. On the other hand, the spectral properties of chlorophylls in natural waters have been examined more closely (Gitelson 1992; Rundquist et al. 1996; Schalles et al. 1998). These studies reveal consistent absorption by algal chlorophylls in the red bandwidth of the electromagnetic spectrum (600-700 nm), which broadly parallels the spectral behavior of chlorophylls in higher plants (Gitelson and Merzlyak 1997; Richardson et al. 2002) and bryophytes (Lovelock and Robinson 2002). Similarly, red absorption is a key metric used in the remote sensing of phytoplankton (Richardson 1996) and macroalgae (Guillaumont et al. 1997) in marine environments.

The present study builds on these observations by assessing a new application of visible-near-infrared (VNIR) reflectance spectroscopy: the prediction and reconstruction of sedimentary chlorophyll concentrations in lake sediments. Specifically, we explore the use of simple reflectance indices to infer concentrations of sedimentary chlorophyll *a* and its derivatives for a set of mountain lakes, exploiting *a priori* knowledge of the absorption spectra of these pigments (Gitelson 1992; Rundquist et al. 1996; Schalles et al. 1998, 2000).

MATERIALS AND METHODS

Study sites and sediment cores

The primary materials used in this study are sediments from four lakes situated in Rocky Mountain National Park (RMNP), Colorado Front Range, U.S.A. (Fig. 2.1). Sediments from a fifth site, in the Mount Zirkel Wilderness Area approximately 80 km to the northwest, are used to validate observations from the other four lakes. The study sites are typical mid-continental alpine lakes characterized by clear ($Z_{Secchi} > 5$ m), chemically dilute (conductivities < 10 $\mu S \cdot cm^{-1}$), and slightly acidic (pH 6.2-6.5) waters. The lakes have short ice-free seasons (July to October). All five catchments occupy high elevation cirques (3200-3400 m above sea level) of last glacial (Pinedale) age and are situated at or slightly above alpine treeline. The dominant catchment substrates are bedrock (Precambrian granites, gneisses and quartz monzonites) and talus derived from these lithologies. Although the lakes are oligotrophic (TP < 0.01 $mg \cdot L^{-1}$), inorganic nitrogen concentrations ($NO_3^- + NH_4^+$) are well-above natural background levels (Table 2.1).

Short (22-40 cm total length) undisturbed sediment cores were obtained from the deepest midpoint of each lake basin using a modified Kajak-Brinkhurst apparatus (Glew 1989), and extruded in consecutive 0.5-cm or 1.0-cm increments once returned to shore (Glew 1988). Spectral and pigment analyses were conducted on 23 core depths from the RMNP lakes (Lake Louise: 8 samples; lakes Husted, Snowdrift and Nokoni: five samples each). VNIR reflectance spectra were obtained from an additional 17 depths in the Pristine Lake core. These samples include both pre- and post-1950 sediments from each lake. Each core has been dated using excess ^{210}Pb activities determined by α spectroscopy, to which the constant rate of supply (CRS) model has been applied (Wolfe

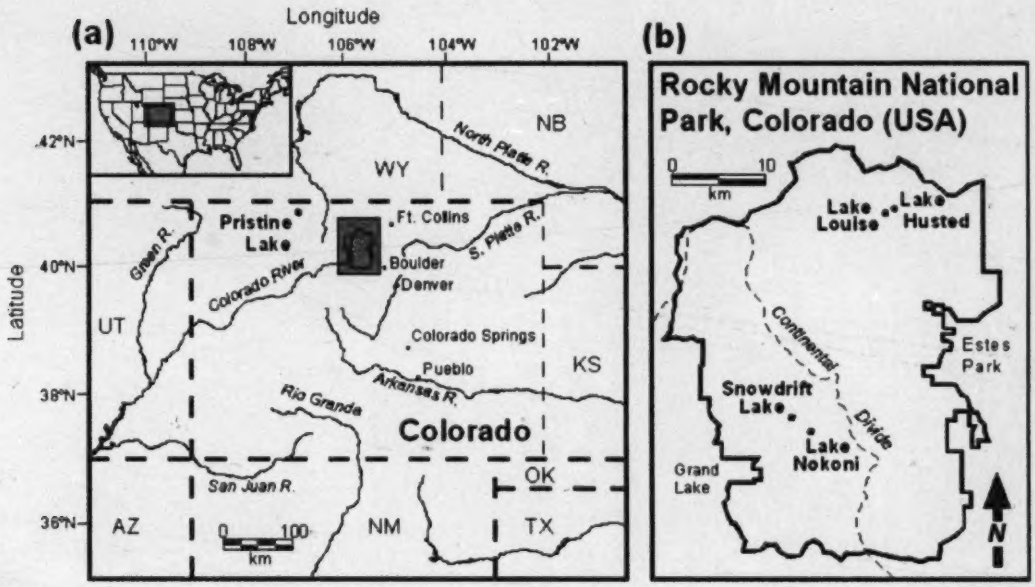

Fig. 2.1. Location maps of the investigated sites in the Colorado Rocky Mountains, showing (a) Pristine Lake, Mount Zirkel Wilderness Area, in relation to Rocky Mountain National Park, and (b) the four study lakes located in Rocky Mountain National Park.

Table 2.1. Site characteristics and water chemistry of the studied lakes.

Lake Name	Latitude North	Longitude West	Elevation (m a.s.l.)	Area (ha)	Depth (Z_{max}; m)	$[NO_3^-]$ (all nutrients in $mg \cdot L^{-1}$)	$[NH_4^+]$	[TP]
Rocky Mountain National Park								
Louise	40°30′28″	105°37′13″	3360	2.7	8	1.026	0.031	0.009
Husted	40°30′56″	105°36′50″	3350	4.0	11	0.267	0.033	0.005
Snowdrift	40°20′30″	105°44′11″	3390	3.2	14	0.107	0.015	0.004
Nokoni	40°15′15″	105°43′50″	3290	7.6	38	0.078	0.023	0.003
Mount Zirkel Wilderness Area								
Pristine	40°41′27″	107°40′53″	3370	2.4	9	0.658	0.019	0.011

et al. 2003). Because our objective is to focus on stable inventories of sedimentary algal pigments, samples from the uppermost 1.5 cm of the cores were not analyzed. This represents the zone of most intense sediment redox cycling, and hence of maximum potential chemical instability among pigments (Leavitt and Hodgson 2001).

The recent paleolimnology of this region has been examined in considerable detail (Baron et al. 2000; Wolfe et al. 2001, 2002, 2003), revealing a suite of recent ecological and biogeochemical changes that reflect initial ecosystem responses towards enhanced anthropogenic deposition of fixed nitrogen from industrial, agricultural, and automotive sources. These stratigraphic shifts, which include increased representations of mesotrophic diatom taxa (e.g., *Asterionella formosa*), declining sediment C:N and $\delta^{15}N$ values, and greater autochthonous organic matter production, have accelerated markedly since approximately 1950 AD.

Sediment spectral reflectance

For the analysis of sediment spectral reflectance, small (0.2-1.0 g dry mass) freeze-dried samples were first ground (agate mortar and pestle) then sieved (< 125 μm). Samples were stored in the dark at 4 °C following collection. Reflectance spectra were measured with a FieldSpec Pro® spectroradiometer (Analytical Spectral Devices Inc., Boulder, Colorado, U.S.A.) over the 350-2500 nm range. The VNIR portion of the spectrum (350-1050 nm) was quantified using a 512-channel silicon photodiode array overlain with an order separation filter. Each channel, an individual detector itself, is geometrically positioned to receive light within a narrow (approximately 1.4 nm) bandwidth. Light was brought to the instrument using a Lowel Pro® lamp with an

effective view of approximately 45°. Working distance to the sample surface was ~16.5 cm. Light energy was collected though bundled optical fibers with a conical view of ~15°. Reflectance was standardized to a white reference panel (spectralon) between each measurement. Percent reflectance (R) was computed as $(R_{sample}/R_{reference}) \times 100$ for the spectral region of greatest interest: 450 – 900 nm.

Pigment analysis

Sedimentary pigment concentrations were quantified using reverse-phase high pressure liquid chromatography (HPLC; Vinebrooke et al. 2002). Pigments were extracted by soaking freeze-dried sediments in acetone, methanol, and deionized water (80:15:5 by volume) for 24 hours in darkness at 10 °C. Extracts were then filtered (0.2-μm pore nylon), dried under N_2, and stored in the dark at –20 °C until analysis. Dry extracts were reconstituted with an injection solution (70% acetone: 25% ion-pairing reagent: 5% methanol) containing Sudan II (3.2 $mg \cdot L^{-1}$) as the internal reference. The ion-pairing reagent (IPR) consisted of 0.75 g tetrabutyl ammonium acetate and 8.00 g ammonium acetate in 100 mL deionized water. Pigments were separated on an HP 1100 HPLC (Hewlett-Packard Ltd., Mississauga, Ontario, Canada) equipped with a Rainin 200 C18 column (10 cm length, 5 μm particle size). Pigments were detected with an in-line diode array detector (435 nm detection wavelength) and a fluorescence detector (435 nm excitation wavelength, 667 nm detection wavelength). Analytical separation included isocratic delivery (1.0 $mL \cdot min^{-1}$) of mobile phase A (10% IPR in methanol) for 1.5 minutes, a linear succession to 100% solution B (27% acetone in methanol) for 7 minutes, and isocratic retention for 12.5 minutes. The column was re-equilibrated

between samples by continuous isocratic delivery for 3 minutes, a linear return to 100% solution A over 3 minutes, and a final isocratic delivery for 4 minutes. Pigment concentrations were quantified using equations derived from dilution series of authentic standards from the US Environmental Protection Agency (USEPA, National Exposure Research Laboratory, Cincinnati, Ohio), or commercially available standards (Sigma Chemicals Ltd.). All pigment concentrations are expressed as their mass per gram organic matter ($mg \cdot g^{-1}$ OM), which was determined by mass loss on ignition at 550 °C for 2 hours (Heiri et al. 2001). Normalization of pigment concentrations to organic content eliminates any potential biasing from dilution by inorganic sediment constituents (Leavitt 1993; Vinebrooke et al. 2002).

Spectral indices and regression

From the sediment reflectance spectra obtained for the 23 samples that were also analyzed for pigments, seven indices were calculated to summarize the large volume of spectral information. The selection of wavelengths used in defining the following indices is based on comparative observations of the general patterns of R observed in both time frames (e.g., Fig. 2.2). The first two indices are the amplitudes (maximum-minimum) of R shifts between 645 nm and 675 nm, and between 675 nm and 750 nm. The third and fourth indices are ratios of reflectance between the same wavelengths: R_{645}/R_{675} and R_{675}/R_{750}. The fifth and sixth indices are based on the first derivative of R (R′): both the value of R′ at 675 nm and amplitude of R′ between 660 nm and 690 nm were calculated. The final index is the dimensionless area between a straight line joining R values at 600 nm and 760 nm and the underlying curve; this trough in R is recognized in all spectra.

Univariate linear regression analyses were performed using each of the spectral indices as an explanatory variable of the following representative fractions of HPLC-derived pigment concentrations: chlorophyll *a* + chlorophyll *a* isomers (i.e., primary chlorophyll *a*), pheophytin *a* + pheophorbide *a* (pheopigments from chlorophyll *a* degradation), and the sum of these two fractions (primary and degraded chlorophyll *a*). All regressions were performed with SPSS v.10.0 (Norusis 2000).

RESULTS AND DISCUSSION

Spectral characteristics of RMNP lake sediments and an algal culture

The most prominent feature of VNIR reflectance spectra obtained from RMNP lake sediments is a trough centered on 675 nm that is attributed to the presence of chlorophylls. This trough is seen in both a green algal culture consisting of *Chlorella* (Schalles et al. 1998) and lake sediments (Fig. 2.2a, b). We confirmed that the reflectance trough in the lake sediments was solely attributable to chlorophyll by organic extraction of the pigment and its derivatives.(Vinebrooke et al. 2002), and subsequent VNIR analysis of the dried sediment residue, which no longer retained a reflectance trough at 675 nm. Therefore, differences between the spectral signatures of chlorophyll-amended water (Fig. 2.2a) and lake sediments (Fig. 2.2b) arise from the markedly different reflectance properties of the media (aqueous versus particulate) in which chlorophyll is diluted, rather than the spectral behavior of chlorophyll itself. The other difference between the algal and sedimentary spectral signatures around 550 nm (Figs. 2.2a, b) is likely attributable to accessory non-chlorophyll pigments contained in the algal cells, given that HPLC analyses revealed relatively low carotenoid concentrations in

sediments. Furthermore, the development of this trough does not appear related in any way to background values of R, which progressively increases between 450 and 900 nm. Between-lake differences in the values of R (Fig. 2.2b) likely reflect subtle variations in the reflective properties of sediment mineral components.

Comparison of recent and old sediments from Lake Louise (Figs. 2.2c, d) and the other lakes (Fig. 2.3) showed that chemical changes associated with algal death, sedimentation, and decomposition did not induce substantial shifts in the wavelength of minimum red reflectance. Indeed, pre- and post-1950 sediments from the four mountain lakes exhibited reflectance spectra that are very similar, except for the 675-nm trough, which is more pronounced following 1950 (Fig. 2.3). Although the preservation of chlorophyll reflectance features in sediment has been recognized for some time (Schalles et al. 2000), these have not previously been calibrated to quantitative sedimentary pigment concentrations.

Fossil pigment stratigraphy

The organic content of sediments from the four RMNP lakes varies between 10% and 35%, and, with the exception of Lake Husted, increases towards the mud-water interface. Chlorophyll *a* and associated pheopigments are consistently preserved in all samples analyzed. For this reason, and given that our primary interest lies in whole-lake algal production, only chlorophyll *a* and its degradation products are considered further. With few exceptions, the stratigraphic trend in concentrations of these pigments is an increase towards the surface of each of the analyzed cores (Fig. 2.4). Concentrations of total sediment chlorophyll *a* and derivatives range from < 0.1 $mg \cdot g^{-1}$ OM in pre-1950

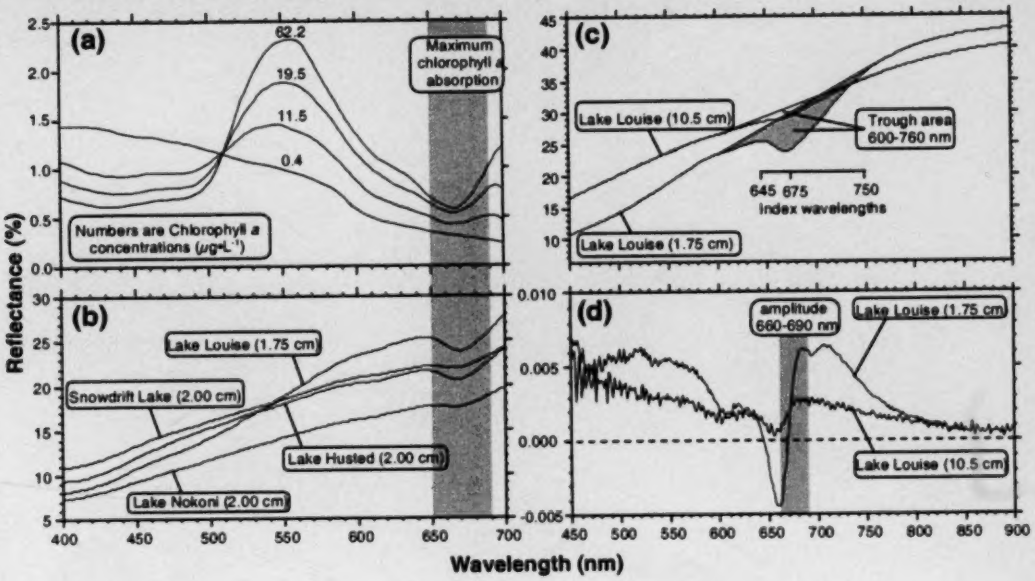

Fig. 2.2. Comparison of reflectance spectra (%R from 400-700 nm) between (a) chlorophyll *a* dilutions of lake water with living *Chlorella* cells (Schalles et al. 1998) and (b) the upper lake sediment samples from the present study. The region of chlorophyll absorption is clearly identifiable in both examples. In (c), representative spectra (%R from 450-900 nm) are illustrated for the most recent (1.75 cm, ~1990) and oldest (10.0 cm; pre-industrial) sediments from Lake Louise. The first derivative of these spectra is shown in (d). In both (c) and (d), the key wavelengths used in defining spectral indices for chlorophyll prediction are indicated.

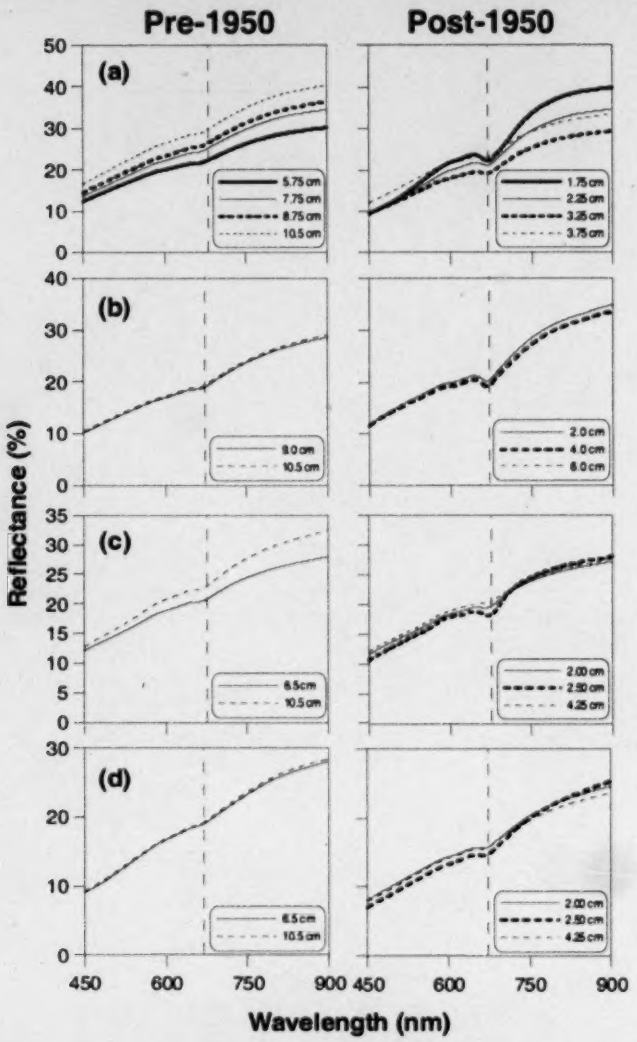

Fig. 2.3. Visible-near-infrared (VNIR) reflectance spectra (450-900 nm) of sediments from four lakes in Rocky Mountain National Park: (a) Lake Louise, (b) Lake Husted, (c) Snowdrift Lake, and (d) Nokoni Lake, subdivided as pre- and post-dating ~1950 AD, as determined by sediment ^{210}Pb activities and constant rate of supply (CRS) depth-age models (Wolfe et al. 2003). Dashed vertical lines indicate 675 nm.

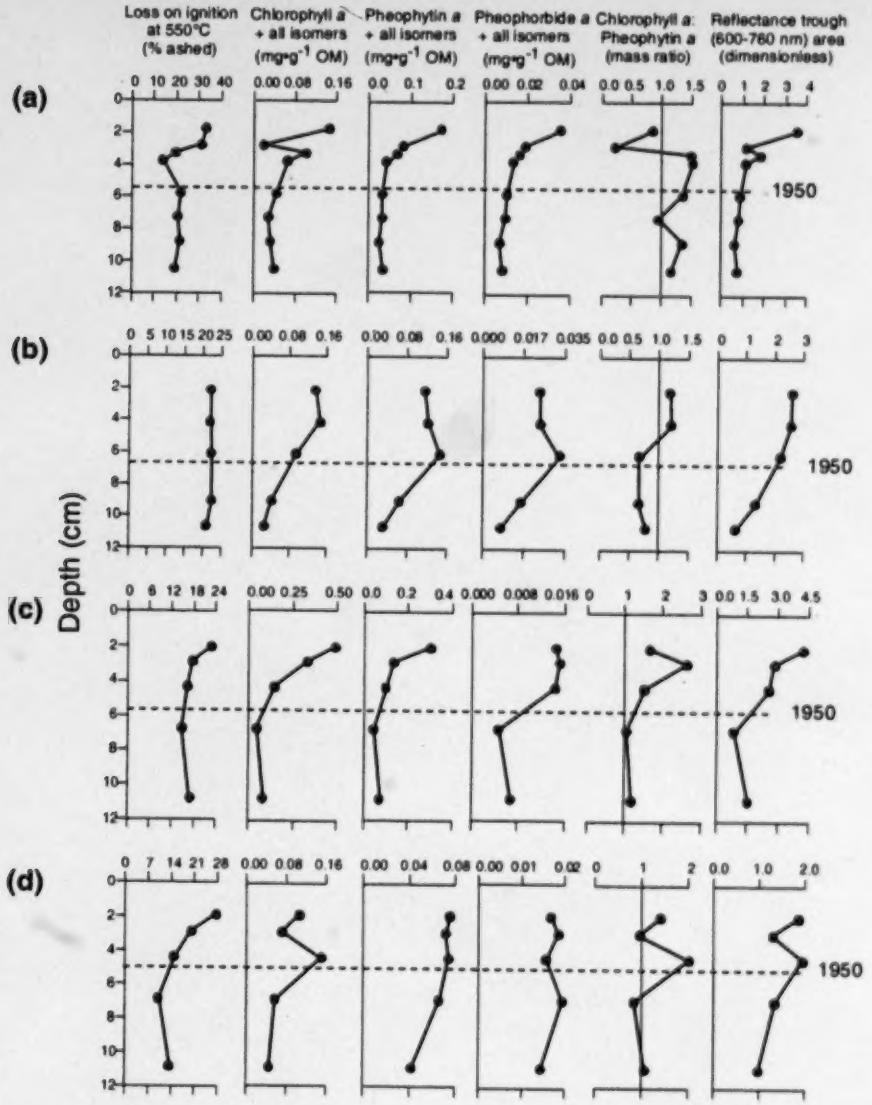

Fig. 2.4. Stratigraphies of organic content, selected pigment concentrations, and related parameters from four lakes in Rocky Mountain National Park: (a) Lake Louise, (b) Lake Husted, (c) Snowdrift Lake, and (d) Nokoni Lake. At right is the area of the reflectance trough between 600 nm and 760 nm, the most robust predictive index of sediment chlorophyll concentrations.

sediments, to a maximum of 0.9 $mg \cdot g^{-1}$ OM at the surface in Snowdrift Lake. These values are typical for oligotrophic lake sediments, as is the relatively large degree of between-lake variability in sediment pigment concentrations (Vinebrooke et al. 2002). Clearly, neither the production nor the preservation of algal pigments is uniform across the sediment surface, and this mandates a critical assessment of the possibility that consistent between-lake stratigraphic trends (Fig. 2.4) are merely preservational artifacts.

An appropriate approach to assessing diagenesis is by comparing chlorophyll *a* concentrations to those of its principal degradation product, pheophytin *a* (Vinebrooke et al. 2002). Our data do not reveal high pheophytin *a* concentrations in samples of low chlorophyll *a* concentration, the expected pattern if diagenetic processes were modulating the down-core distribution of pigments. Indeed, the chlorophyll *a*: pheophytin *a* ratio does not show any consistent stratigraphic trends among the four lakes (Fig. 2.4). Pheophorbide *a* concentrations, which have widely been interpreted as proxies for algal grazing by invertebrates (e.g., Leavitt et al. 1994) are several-fold lower than those of pheophytin *a* in RMNP lake sediments. However, both pheopigments have very similar down-core stratigraphies (Fig. 2.4). This does not support the regulation of pheophorbide *a* as uniquely a by-product of grazed algal standing crop, all the more since the four lakes have markedly different fish stocking histories (Wolfe et al. 2003), which constitutes the dominant regulator of invertebrate herbivory. The production of pheophorbide *a* in the absence of grazing has been recognized in marine ecosystems (Head et al. 1994), and can therefore be suspected for lakes. Thus, the stratigraphic profiles of both pheopigments can be simply attributed to greater sources of reactive chlorophyll *a* in the most recently deposited sediments, in turn due to increased algal standing crop. Although pigment

degradation is clearly occurring in the sediments of the RMNP lakes (incompleteness of carotenoid record, abundance of pheopigments), this does not appear to obscure the primary environmental signal suggested by the pigment stratigraphy: progressive increases in algal production and sedimentation over recent decades.

Regression between spectral indices and sedimentary pigments

For the 23 sample populations, seven reflectance indices were regressed against three categories of sedimentary pigments: chlorophyll *a* and its isomers; pheopigments from chlorophyll *a* degradation (pheophytin *a* + pheophorbide *a*); and the sum of the first two classes (hereafter: chlorophyll *a* + derivatives). Of the indices, only two failed to produce significant correlations (the first derivative value at 675 nm and the amplitude of reflectance change between 645 nm and 675 nm). All other spectral indices were significantly ($p < 0.05$) correlated to all three categories of sedimentary pigment concentrations expressed per unit organic matter (Table 2.2). These correlations do not differ appreciably when sedimentary pigment concentrations are expressed per unit dry mass of sediment. The three most highly correlated indices are: reflectance trough area between 600 nm and 760 nm, the amplitude of R between 675 nm and 750 nm, and the amplitude of R´ between 660 nm and 690 nm. The regressions obtained from these three indices and the three pigment categories are illustrated (Fig. 2.5).

Interestingly, spectral indices are more closely correlated to the sum of pheopigments than to their precursor, chlorophyll *a*. However, the correlation coefficients are similar for both pigment classes (Table 2.2), which show that both chlorophyll and its degradation products have similar reflectance properties. This implies that diagenesis of

Table 2.2. Correlation coefficients (r) for linear regressions between various spectral indices and sediment pigment concentrations.

Spectral index	Fraction of sedimentary pigments		
	Chlorophyll *a* + isomers	Pheophorbide *a* + pheophytin *a*	Chlorophyll *a* + derivatives
Reflectance trough area 600-760 nm	0.84**	0.94**	0.92**
Amplitude of reflectance 675-750 nm	0.74*	0.88**	0.83**
Amplitude of first derivative 660-690 nm	0.66*	0.86**	0.82**
Ratio of reflectance 645/675 nm	0.63*	0.67*	0.68*
Ratio of reflectance 675/750 nm	-0.57*	-0.71*	-0.68*
First derivative value at 675 nm	-0.39	-0.58	-0.56
Amplitude of reflectance 645-675 nm	0.27	0.44	0.38

** $P < 0.01$; * $P < 0.05$

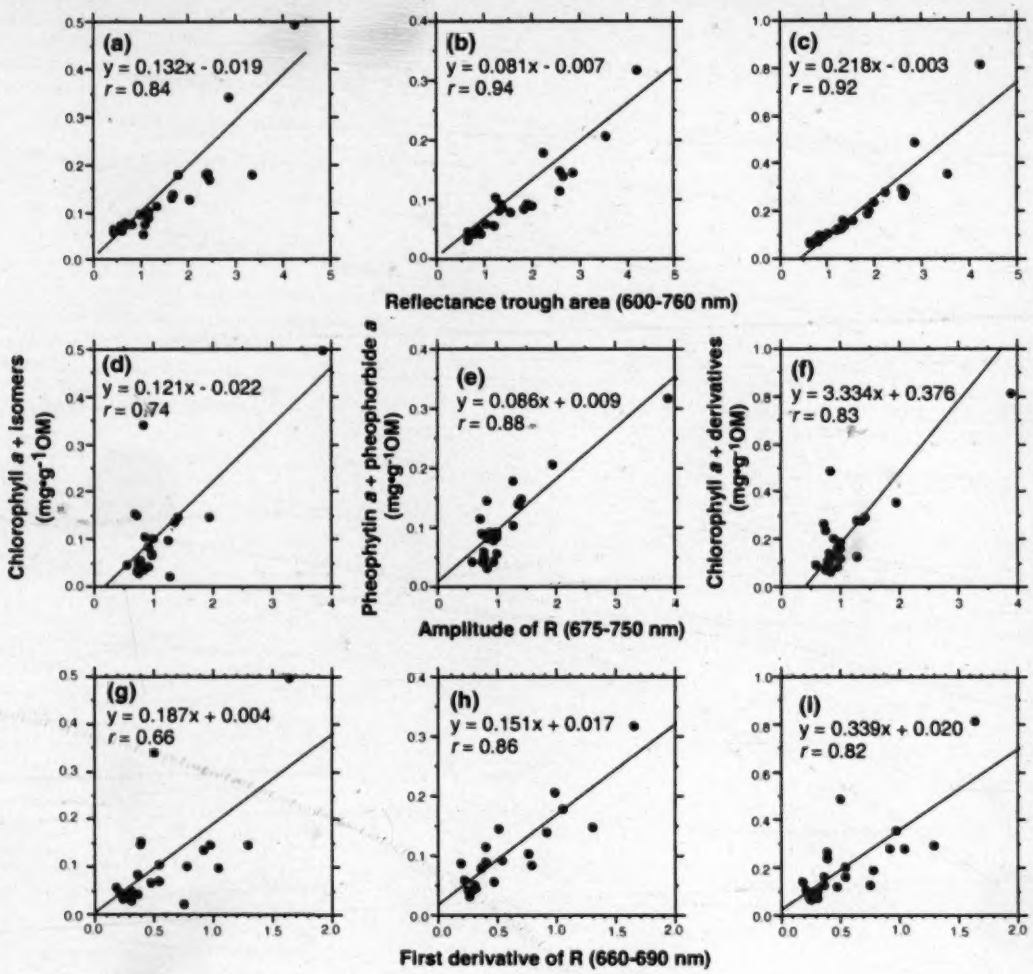

Fig. 2.5. Regressions (n = 23) between the three most highly predictive spectral indices and representative categories of sedimentary pigments: (a-c) reflectance trough area (600-760 nm); (d-f) amplitude of raw reflectance (675-750 nm); and (g-i) amplitude of the first derivative of reflectance (660-690 nm).

sediment organic matter is unlikely to significantly alter the portions of reflectance spectra that relate most strongly to algal production (i.e., 600-760 nm).

Development of predictive models

The strength of the above relationships allows the development of simple inference models based on the three most statistically robust spectral predictors of sedimentary pigments. These models are directed towards the reconstruction of the most inclusive pigment category, chlorophyll *a* + derivatives, which is believed to best reflect total algal abundance and hence be of greatest potential utility for reconstructing lake trophic evolution. Linear regressions developed between spectral indices and pigment categories were applied to the 23 reflectance spectra, and measured *versus* inferred values of chlorophyll *a* + derivatives were graphed (Fig. 2.6). The total integrated area of the reflectance trough from 600-760 nm was the best predictor of three metrics of sedimentary chlorophyll concentrations (Table 2.2). This index was particularly successful in producing a cluster of points closely surrounding the 1:1 line at concentrations < 0.3 $mg \cdot g^{-1}$ OM, which is also shown by reduced scatter and trend in residuals, by comparison to the other two indices (Fig. 2.6). Although more work is clearly needed for sediments with higher chlorophyll *a* concentrations, our analyses show that simple spectral indices are potentially sensitive to changes of sediment chlorophyll *a* + derivatives in the order of $\pm$ 0.15 $mg \cdot g^{-1}$ OM.

Another way of depicting this data is to plot stratigraphically both measured and inferred values alongside each other (Fig. 2.7). This enables the largest mismatches to be readily identified, for example the model's over-estimation at the top of the Lake Louise

core, and under-estimations in the two most recent samples from Snowdrift Lake. However, with the exception of these points, the reflectance trough index faithfully reproduces measured sediment pigment concentrations with remarkable degrees of accuracy and precision. This implies that overall trends in sedimentary chlorophyll concentrations may be reconstructed in the absence of HPLC measurements. However, the inferred values shown here (Fig. 2.7) do not constitute reconstructions in the strict sense, because they represent the same samples used in the inference model's development (Fig. 2.6). To avoid this element of circularity, we now apply the model to a fifth lake outside the calibration set, and for which there exist several independent lines of evidence for increased production.

Application to lake trophic reconstruction

Pristine Lake receives significant amounts of nitrogen deposition originating from coal-fired power plants in the lower Yampa River valley, notably at Hayden and Craig (Turk and Campbell 1987). These emissions are transported to the Mount Zirkel Wilderness by prevailing westerly winds. Inorganic N deposition rates in the region thus exceed 4 kg $N \bullet ha^{-1} \bullet y$ (Williams and Tonnessen 2000). This is directly comparable to RMNP lakes east of the Continental Divide, which are affected by N emissions from the Front Range urban corridor (Baron et al. 2000).

Sediments from Pristine Lake record a large increase in total diatom concentrations since 1950, associated with increased abundances of the mesotrophic planktonic taxa *Asterionella formosa* and *Fragilaria tenera* (*Synedra sensu lato*) (Fig. 2.8a). Sedimentary chlorophyll *a* + derivatives was inferred by NIRS for 17 intervals in

the Pristine Lake core, using the model derived from reflectance trough area (600-760 nm). This reconstruction yields estimates ranging from < 0.05 $mg \cdot g^{-1}$ OM before 1950, to values up to 0.4 $mg \cdot g^{-1}$ OM in the last decade (Fig. 2.8b). Reflectance-inferred increases in chlorophyll concentrations were correlated significantly with other proxies of lake enrichment, including relative frequencies of *Asterionella formosa* ($r = 0.75$, $p < 0.01$) and *Fragilaria tenera* ($r = 0.82$, $p < 0.01$), as well as organic carbon content ($r = 0.88$, $p < 0.01$). These changes (Fig. 2.8a) are directly comparable to those documented from five RMNP lakes, including those considered previously here (Wolfe et al. 2003). Moreover, the inferred sedimentary chlorophyll stratigraphy matches the chronology of regional energy development and population growth exceptionally well.

It is interesting to note that the parallel behavior of diatom and inferred sedimentary chlorophyll concentrations observed before 1975 appears to collapse in subsequent decades. This may suggest the progressive replacement of diatoms by non-siliceous algal groups, as resource ratios continue to shift. Irrespective of these finer points of interpretation, which remain to be confirmed, the data unequivocally support the contention that VNIR spectral data may produce realistic reconstructions of trends in sedimentary chlorophyll concentration, which is a more integrative measure of whole-lake production relative to proxies such as diatom concentrations (Vinebrooke et al. 2002). In the case of Pristine Lake, the remotely-sensed data corroborate diatom-based inferences of recent nutrient enrichment, together providing a powerful case that this lake has markedly deviated from its natural, pre-industrial, trophic state.

In summary, the results of this investigation confirm that reflectance spectroscopy is a powerful tool in paleolimnology (Korsman et al. 2001). Specific regions of sediment

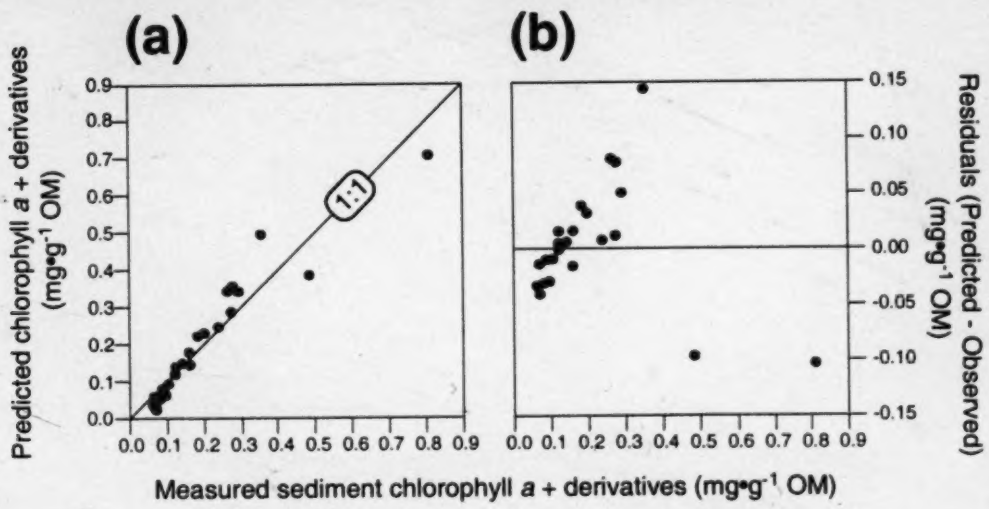

Fig. 2.6. (a) Comparison of measured (by HPLC) and inferred total concentrations of chlorophyll *a* + derivatives obtained using the regression of Fig 5a, and (b) plot of corresponding residuals.

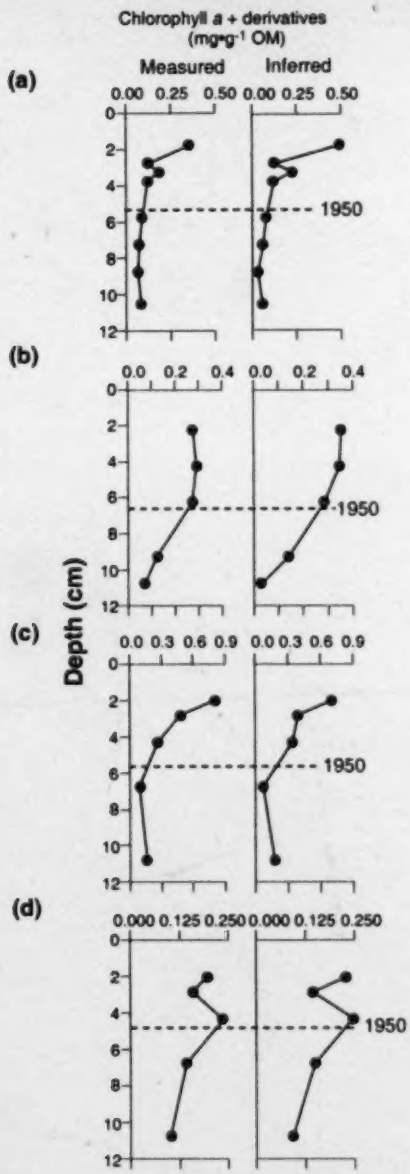

Fig. 2.7. Down-core comparisons of measured and inferred sediment concentrations of total chlorophyll *a* + derivatives derived for (a) Lake Louise, (b) Lake Husted, (c) Snowdrift Lake, and (d) Nokoni Lake. Inferred values are based on the reflectance trough area index (600-760 nm).

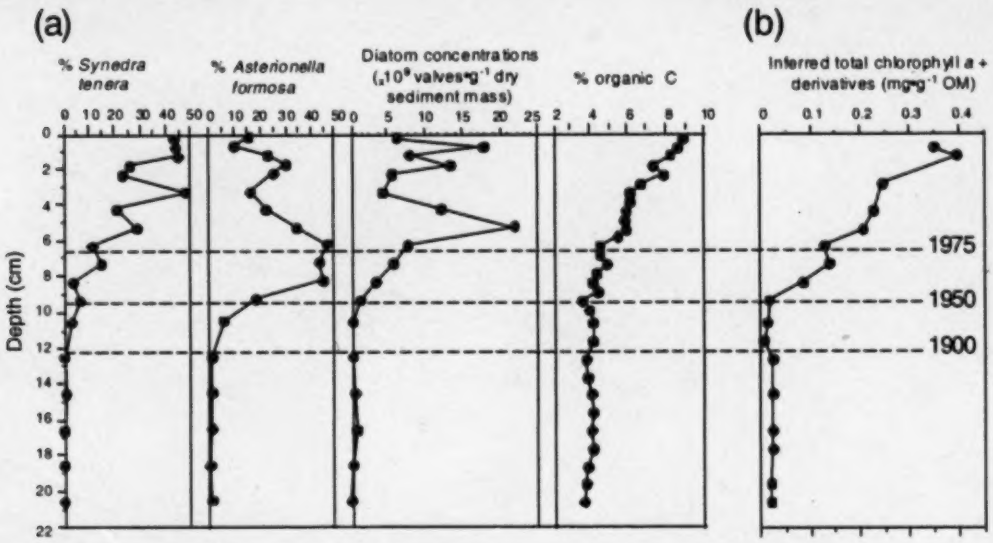

Fig. 2.8. Conventional paleolimnological proxies and corresponding ^{210}Pb ages for Pristine Lake in the Mount Zirkel Wilderness Area (a), shown alongside reflectance-based estimates of total sediment chlorophyll *a* + derivatives, inferred from the reflectance trough area index (b). Protocols for diatom analysis, organic carbon, and dating are described elsewhere (Wolfe et al. 2003).

reflectance, notably in the red portion of the spectrum, demonstrably relate to sedimentary concentrations of chlorophyll *a* and its degradation products. Simple models can be developed to infer sediment photosynthate concentrations, and thereafter be applied to the reconstruction of changing lake trophic regimes. This method does not supplant wet chemical methods, which remain necessary for the quantification of trace pigments such as carotenoids. Rather, VNIR methods enable rapid and non-destructive semi-quantitative assessments of bulk sediment chlorophyll concentrations. For many paleolimnological applications, including studies of eutrophication (Hall and Smol 1999) and animal-derived nutrient fluxes (Finney et al. 2000), this information may nonetheless be critical.

Measured pigment concentrations associated with total primary production (i.e., chlorophyll *a* and its derivatives) have increased over recent decades in all four investigated RMNP lakes, in agreement with additional proxies such as diatoms, nitrogen stable isotopes, and organic matter provenance (Wolfe et al. 2002, 2003). The results from Pristine Lake in the Mount Zirkel Wilderness Area confirm that recent nutrient enrichment is widespread in the Colorado Rocky Mountains. Similar observations have now also been documented from the Beartooth-Absaroka Wilderness of Montana and Wyoming (Saros et al. 2003), as well as from Sequoia and Yosemite National Parks in the Sierra Nevada (Sickman et al. 2003). All of these regions are congressionally-designated Class 1 wildernesses under the Clean Air Act, which, in principle, specifically mandates protection from human-induced ecological changes. Nonetheless, recent observations collectively constitute a mounting body of evidence indicating that mid-continental alpine lakes are changing directionally in response to increased nutrients

transported atmospherically from a variety of anthropogenic sources.

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CHAPTER THREE: Visible-near-infrared (VNIR) spectral characterization of chlorophyll in lake sediments

INTRODUCTION

Lake sediments integrate a variety of organic and inorganic constituents. Fossil pigments, one of the preserved organic components, are a useful proxy for reconstructing lake primary production, algal competition, and invertebrate herbivory (Hurley and Armstrong 1991; Leavitt et al. 1994; Leavitt and Hodgson 2001). The chlorophylls are the most abundant pigment found in most lake sediments. One of the confounding issues when interpreting lake sediment pigment profiles is the degradation of chlorophyll *a*, which may be rapid in certain instances (Cuddington and Leavitt 1999). The two primary chlorophyll *a* degradation pathways are loss of Mg^{2+} from the tetrapyrole ring, resulting in pheophytin *a*, and loss of the phytol chain, which produces pheophorbide *a*. Accelerated degradation of chlorophyll *a* in the natural environment occurs by exposure to sunlight, high temperatures, and oxidizing conditions (Leavitt and Hodgson 2001). In extreme cases, complete remineralization of chlorophyll *a* (ultimately to CO_2 and H_2O) may occur (Hörtensteiner 1999; Matile et al. 1999; Takamiya et al. 2000). Both pre- and post-depositional processes are important in modulating sedimentary pigment preservation (Leavitt 1993; Steenbergen et al. 1994; Sinninghe-Damsté and Koopmans 1997; Hodgson et al. 1998). After deposition, fossil pigments may be altered by microbial activity (Leavitt and Carpenter 1990a, 1990b), which further contributes to diagenesis (Hurley and Armstrong 1990). However, pigment degradation rates are typically much lower under anoxic conditions (Hurley and Armstrong 1990).

A range of analytical techniques has been developed for quantifying pigment concentrations in lake sediments. Currently, the most widely used method is reverse-phase high-pressure liquid chromatography (rp-HPLC, hereafter HPLC). Although the non-destructive technique of visible-near infrared (VNIR) reflectance spectroscopy has been used to detect algal pigments in natural waters (Gitelson 1992; Mittenzway et al. 1992; Rundquist et al. 1996; Schalles et al. 1998, 2000), and to infer foliar health and leaf biochemistry in terrestrial plants (Sims and Gamon 2002), the method has only recently been applied to sedimentary pigments preserved in lake cores (Das et al. 2004). In foliar VNIR studies, indices such as the normalized difference vegetation index [NDVI = $(\text{Reflectance}_{\text{near-infrared}} - \text{Reflectance}_{\text{visible}})/(\text{Reflectance}_{\text{near-infrared}} + \text{Reflectance}_{\text{visible}})$] have proven especially popular (Sims and Gamon 2002), whereas the ratio of near-infrared to visible reflectance has been applied to algal chlorophyll (Gitelson 1992, 2004; Mittenzway et al. 1992). All of these studies have shown that chlorophylls absorb strongly in the blue and red ranges of the electromagnetic spectrum, producing distinctive troughs in reflectance spectra due to absorption near 440 nm and 675 nm (Gitelson 1992; Mittenzway et al. 1992; Rundquist et al. 1996; Schalles et al. 1998, 2000). Chlorophyll absorption in the blue portion of the spectrum (~400-500 nm) overlaps with that of carotenoid pigments (Yacobi et al. 1995), and thus there is a distinct advantage to using red wavelengths (~600-700 nm) for studying absorption due solely to chlorophyll *a* (Yacobi et al. 1995).

In the present study, we build upon the preliminary results of Das et al. (2004, Chapter 2), which suggest that recent changes in alpine lake sediment VNIR spectra correspond to recent increases of sediment chlorophyll *a* concentrations, in turn

attributable to lake trophic enrichment from atmospheric deposition of nitrogen. The study presented in Das et al. (2004, Chapter 2) is restricted to one lake type (oligotrophic alpine tarns) and further does not explore the potential impacts of diagenesis on sediment VNIR spectra. The present paper has the following specific objectives: (A) to document the development of chlorophyll-specific VNIR spectral characteristics through controlled chlorophyll amendments to an inorganic sediment matrix; (B) to develop a new calibration between sediment VNIR spectra and chlorophyll *a* (and derivative pheopigments) measured by HPLC; (C) to assess the influences of diagenetic processes on the VNIR spectral properties of lake sediments; and (D) to apply the methodology to lake trophic reconstruction, with the possibility of comparing, *a posteriori*, the VNIR-based reconstruction to HPLC measurements. In addressing these objectives, we demonstrate the suitability of sediment VNIR approaches across a broad spectrum of lake ecosystems.

Experimental Design

Experiment 1: algal inoculation

Pre-industrial lake sediments from Lake Louise (22.5 cm depth, approximately 200 years old; Wolfe et al., 2003) were used as the matrix for the algal dilution series. Lake Louise is located in Rocky Mountain National Park (RMNP), Colorado, USA (Table 3.1). Detailed site descriptions are presented elsewhere (Wolfe et al. 2001, 2003; Das et al. 2004). The algal inoculum used for the dilution series experiment was collected from the bottom of experimental fish-rearing aquaria. The inoculum comprised mixed

Table 3.1. Water chemistry of the investigated lakes. Data from Lake Louise are from Wolfe et al. (2003), and for Lac La Biche from Mitchell and Prepas (1990), representing average surface waters. All other data are original.

Lake name	Latitude North	Longitude West	Depth (Z_{max}; m)	NH_4^+ ($\mu g \cdot L^{-1}$)	NO_3^- ($\mu g \cdot L^{-1}$)	TP ($\mu g \cdot L^{-1}$)	Conductivity ($\mu S \cdot cm^{-1}$)	pH
Louise	40°30'28"	105°37'13"	8.0	31.00	1026.00	9.0	8	6.2-6.5
Deadmoose	52°19'48"	105°10'12"	48.0					
epilimnion				28.83	2.47	47.6	2,477	8.7
hypolimnion				1,654.80	9.87	369.5	2,866	8.2
Waldsea	52°22'12"	105°20'24"	14.5					
epilimnion				44.83	4.58	73.3	2,849	8.9
hypolimnion				1,421.87	4.37	461.5	3,164	8.5
Lac La Biche	54°50'00"	112°00'00"	21.3	33.00	10.00	110.0	288	7.8-8.9

colonial, coccoid, and filamentous cyanophytes, dominated by *Oscillatoria* and *Microcystis* spp. Bulk algal samples were freeze-dried, ground, and mixed with the sediment at the following sediment:algae mass ratios: 5:1, 10:1, 25:1, 50:1, 100:1, 250:1, 500:1, 750:1, and 1000:1. Chlorophyll *a* concentrations, as well as those of associated pheopigments, were also determined on these samples by HPLC, to allow the development of a simple calibration between VNIR spectra and absolute sediment pigment concentrations (described below).

Experiment 2: algal photo-degradation

A series of fresh to sunlight-bleached algal samples (primarily Charophyceae, *e.g., Chara* spp.) were collected from the shoreline of Deadmoose Lake, a saline, meromictic lake, located 100 km east of Saskatoon, Saskatchewan, Canada (Last and Slezak 1986, 1988). The lake water is highly conductive with elevated total phosphorus and ammonium in the hypolimnion (Table 3.1). The algal samples were collected in a transect perpendicular to the shore of the lake, ranging from living biomass to yellow, completely desiccated, algal remains (Figure 3.1). The oldest samples had most likely been exposed since early spring, a period of approximately 3.5 - 4.0 months, having been stranded by seasonal water level lowering. A progressive loss of green coloration was observed, indicating the samples represent a true chronosequence (Figure 3.1). Collections from 7 stations spaced 1 m apart were kept cool and dark, thereby limiting further pigment degradation until returned to the laboratory.

Experiment 3: redox-mediated diagenetic processes

To evaluate the influence of redox conditions on the preservation of lake sediment VNIR spectra, strongly chemically-reduced surface sediments (0 - 3 cm) were collected by gravity corer (Glew 1989) from the monimolimnion of Waldsea Lake, a neighboring meromictic lake to Deadmoose Lake in central Saskatchewan, with comparable limnology and water chemistry (Table 3.1). Because the corer captures the mud-water interface intact as well as overlying hypolimnetic water *in situ*, this water was retained as the medium for subsequent incubations. In the laboratory, a total of 20 fresh wet samples, each approximately 100g wet mass, were transferred to twenty different 50 ml centrifuge tubes. Of them, 16 were incubated with hypolimnetic water (30 ml) under continuous aeration. One oxidized sample was collected every week, and immediately frozen after removing the supernatant water. Four additional samples were incubated with hypolimnetic water in the same way, but completely sealed, without any aeration. Every 4 weeks one of these samples was removed and frozen in the same way. All incubations were conducted in the dark at 4°C to simulate natural lake-bottom conditions. The experimental apparatus is illustrated in Figure 3.2. Because we anticipated greater pigment degradation and hence VNIR spectral changes in the oxidized samples, these were sampled more intensely (every week) in comparison to reduced samples (every 4 weeks). We report only results from the 4 week time-steps, totaling 112 days (*e.g.*, 0, 28, 56, 84, and 112 days).

Figure 3.1. Fresh (dark colored) and photo-degraded (light colored) charophyte algae collected at one meter intervals on a transect perpendicular to the shore of Deadmoose Lake. The arrows indicate sample collection points.

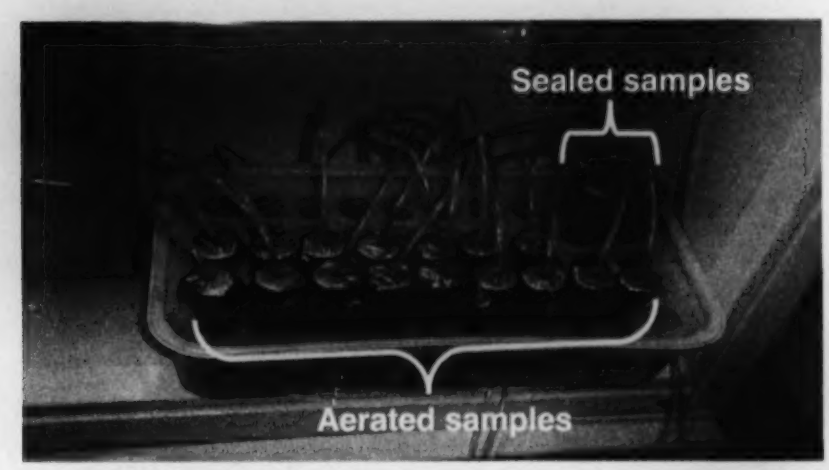

Figure 3.2. Experimental arrangement inside the refrigerator to monitor the impact of oxidation and reduction on sediment VNIR spectral properties. Details are explained in the text.

Application to paleolimnology

The performance of the VNIR chlorophyll inference model developed from the algal dilution series was evaluated through its application to recent sediments from Lac La Biche (Alberta, Canada), collected and extruded in the usual way (Glew 1989), and dated with excess ^{210}Pb (Appleby and Oldfield 1978). Inferred concentrations of total chlorophyll *a* and derivatives were compared to HPLC measurements from the same core levels. Lac La Biche is a large and naturally eutrophic prairie lake that is currently subjected to cultural eutrophication (Mitchell and Prepas 1990).

It is clear that the sediments of Lake Louise (matrix for the dilution experiment), Waldsea and Deadmoose lakes (diagenesis experiments), and Lac La Biche (model validation) all differ markedly with respect to mineralogy and other physical characteristics, owing in part to fundamentally different water chemistries between these lakes (Table 3.1). These elements of variability make it possible to assess the robustness of VNIR-based chlorophyll inferences across a broad limnological gradient. It is hypothesized that chlorophyll *a* absorption features will be similarly expressed across this range of lake types, because chlorophyll *a* is a shared primary photosynthates by all the primary producers inhabiting these environments.

Analytical Methods

VNIR spectral analysis

All samples, once freeze-dried, were ground and sieved though a 125 µm mesh and preserved in the dark prior to VNIR spectral analysis. Spectral measurements were performed with a FieldSpec® Pro (Analytical Spectral Devices, Inc., Boulder, Colorado)

over the range 350 to 2500 nm. A 512-channel silicon photodiode array detector measured the VNIR portion of the spectrum. Each channel is geometrically positioned to receive light within a narrow (approximately 1.4 nm) bandwidth. Light was brought to the instrument using a Lowel Pro Lamp® with an effective illumination view of about 45°, and the working distance to the samples was 16.5 cm. Light energy was collected though a bundle of optical fibers with a conical view of about 15°. White reference was used as the reflectance standard, obtained by measuring reflectance of a spectralon reference panel between each sample. The spectral reflectance collected by the optical fiber sensor of the spectrometer was automatically stored in a notebook computer using the ASD software and subsequently processed to ASCII format for further analysis. All measured spectra between 450 and 900 nm were converted to percent reflectance (%R = $\text{Reflectance}_{\text{sample}} \times 100 / \text{Reflectance}_{\text{reference}}$), and imported to Microsoft Excel for subsequent analysis.

Pigment analysis

Pigment concentration in sediments were detected and quantified with reverse-phase High Performance Liquid Chromatography (HPLC), as described in detail elsewhere (Vinebrooke and Leavitt 1999; Das et al. 2004; Chapter 2). A standard mixture of acetone, methanol, and water (80:15:5, by volume) was used for pigment extraction from sediment samples. Pigment concentrations are expressed as $mg \cdot g^{-1}$ organic matter ($mg \cdot g^{-1}$ OM) using estimates of organic content obtained by loss on ignition (LOI) at 550°C for 2 hours (Heiri et al. 2001). Normalization to sediment organic content is a standard practice in lake sediment pigment analysis, aimed to minimize the effects of

clastic (inorganic) dilution of pigments from those associated with *in situ* degradation of photosynthates (Leavitt and Hodgson 2001). Pigments were analyzed by HPLC for all the samples comprising the dilution series, as well as those from the Lac La Biche sediment core.

Spectral indices and calibration

The choice of spectral indices for calibration is based on wavelengths deemed sensitive from the patterns of VNIR spectral reflectance observed in the algal dilution series samples. We calculated ratios of %R involving both red and near-infrared wavelengths (650/675 nm, 675/750 nm), as well as the amplitudes of reflectance between 650 and 675 nm, as well as between 675 and 750 nm. We also calculated the dimensionless area between a straight line joining R values at 650 nm and 750 nm and the underlying curve; this trough in R is detected in all samples. The reflectance trough area we have selected in this study is different from the previous (Chapter 2; Das et al. 2004) possibly because of an interference spectral feature in the region between 600 and 650 nm not attributed to chlorophyll *a*.

For each of these simple indices, linear regressions were undertaken with concentrations of chlorophyll *a* + associated derivatives (*e.g.*, chlorophyll *a*, chlorophyll *a* isomers, pheophytin *a*, and pheophorbide *a*). SPSS v. 10.0 was used for these regressions (Norusis 2000). We deliberately chose to focus on the most inclusive pigment category (chlorophyll *a* + derivatives), as this is most likely to best reflect whole-lake production (Das et al. 2004, Chapter 2).

RESULTS

Algal dilution series

HPLC analysis of the algal inoculum revealed the presence of the following major pigments: chlorophyll *a*, pheophytin *a*, pheophorbide *a*, *β*-carotene, and the collective category lutein + zeaxanthin (Figure 3.3). Total chlorophyll *a* and derivatives comprised 6.36 $mg \cdot g^{-1}OM$ of the pure inoculum material, and 0.05 $mg \cdot g^{-1}OM$ in the most highly diluted sample (1000:1) of the series. In the pure matrix to which the inoculum was added, pigments were at or below detection limits (<0.01 $mg \cdot gOM^{-1}$), with the exception of relatively high concentrations of total pheopigments (0.034 $mg \cdot gOM^{-1}$) that are associated with the age of this material (~200 years).

The most evident spectral features in the algal inoculum are: (a) low visible reflectance with several minor absorption features; (b) a significant trough in reflectance centered on approximately 675 nm, and (c) much higher overall reflectance in the NIR (Figure 3.4A). The depth of the trough between 650 and 750 nm shallows unidirectionally with incremental dilution of the algal inoculum (Figure 3.4 B). Based on the strength of the relationship between the algal mass added to the dilution series and reflectance trough area in this band ($r = 0.99$, Figure 3.5 A), the trough centered near 675 nm appears directly related to chlorophyll concentrations.

Inference model development

Of the various indices regressed against HPLC-determined pigment concentrations, the most robust predictor is the spectral trough area between 650 and 750 nm (Table 3.2). The amplitude of reflectance between 675 and 750 also yielded

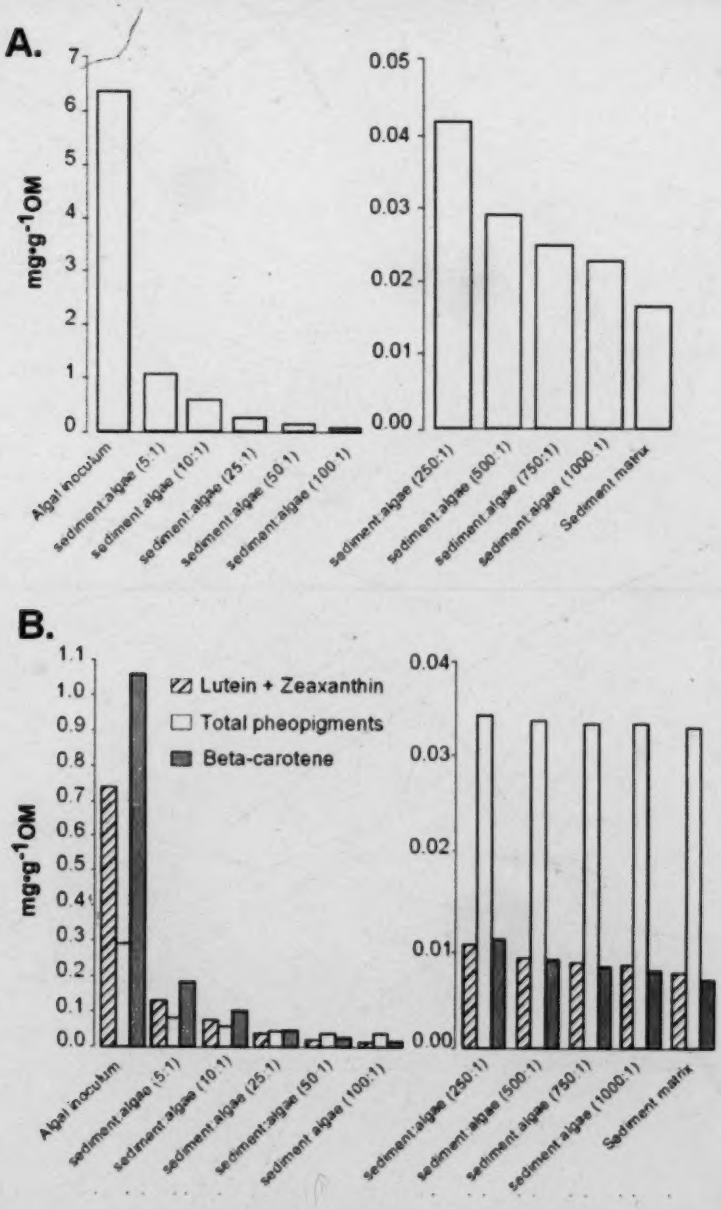

Figure 3.3. Total chlorophyll *a* + isomers (A) and other detected pigments (B) (lutein + zeaxanthin, total pheopigments including pheophytin *a* and pheophorbide *a*, and beta-carotene) measured in the dilution series experiment using high-pressure liquid chromatography (HPLC).

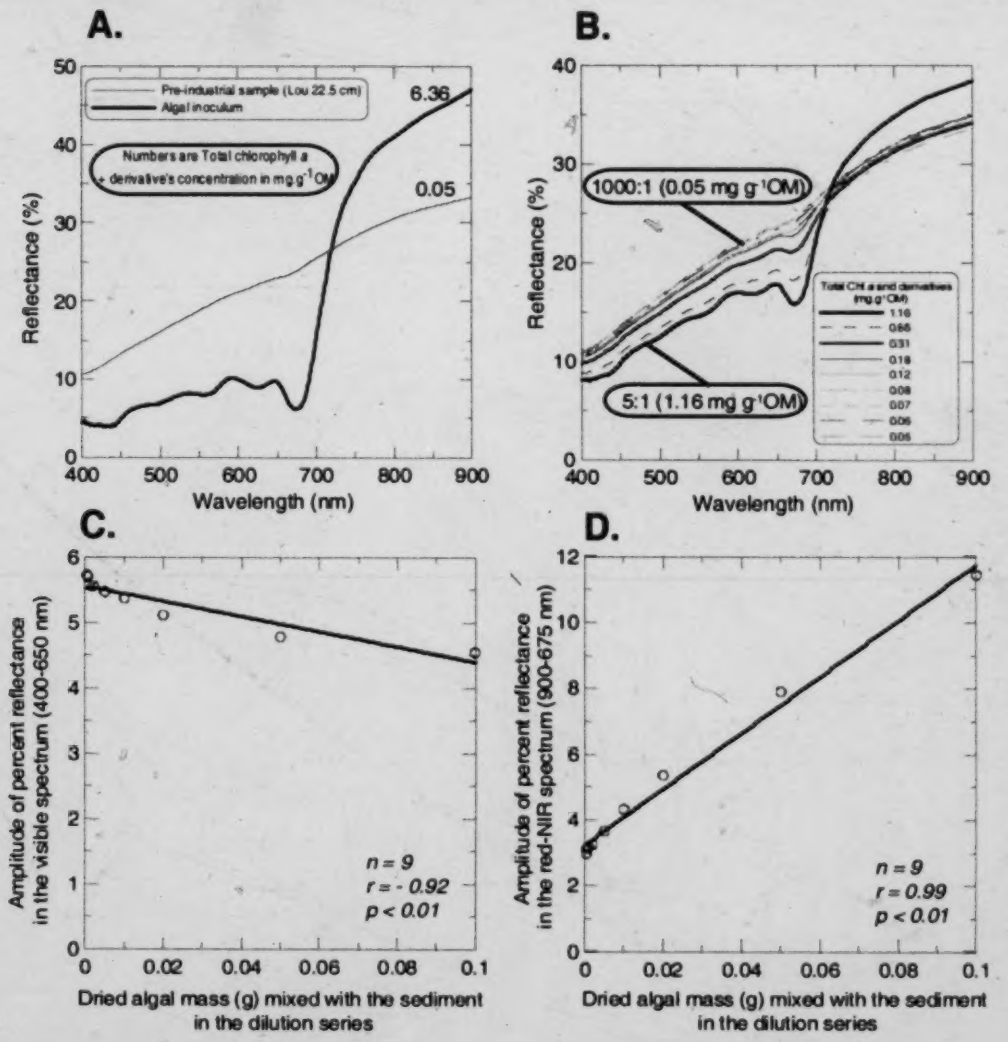
A.
B.
C.
D.
Pre-industrial sample (Lou 22.5 cm)
Algal inoculum
6.36
0.05
Numbers are Total chlorophyll a + derivative's concentration in mg g-1OM
Reflectance (%)
Wavelength (nm)
1000:1 (0.05 mg g-1OM)
5:1 (1.16 mg g-1OM)
Amplitude of percent reflectance in the visible spectrum (400-650 nm)
n = 9
r = - 0.92
p < 0.01
Dried algal mass (g) mixed with the sediment in the dilution series
Amplitude of percent reflectance in the red-NIR spectrum (900-675 nm)
n = 9
r = 0.99
p < 0.01

Figure 3.4. Percent VNIR reflectance (400-900 nm) for (A) the dilution matrix and the algal inoculum; (B) the complete dilution series. In (C), the relationship between the amplitude of blue reflectance (400-500 nm) and dried algal inoculum mass is shown. A similar plot for red-near infrared reflectance (650-750 nm) is shown in (D).

significant correlations. In both cases, these correlations are roughly equal for chlorophyll *a* alone and the sum of chlorophyll *a* and derivative pheopigments, but lower for the sum of pheopigments taken alone. From this linear regression (Figure 3.5 B), it is possible to use the spectral data alone to infer chlorophyll *a* concentrations from the same samples, and then compare inferred and predicted values (Figure 3.6A). This model yields a close cluster around the 1:1 line with generally low residual values that lack any detectable trend (Figure 3.6 B). This dilution series regression was subsequently applied to the various samples used to assess diagenesis, in an attempt to quantify the impacts of degradation on chlorophyll spectral signatures and apparent (*i.e.*, VNIR-inferred) chlorophyll *a* concentrations.

Diagenetic processes and VNIR spectra: sunlight bleaching

The spectra of living, desiccated, and progressively sun-bleached algae collected from the shore of Deadmoose Lake are remarkably similar in shape and amplitude to those of lake sediments (Figure 3.4B; Das et al. 2004). This is perhaps because all the dried samples (*i.e.*, every one except 0 m, which was living algae) contained appreciable quantities of adhered mineral matter. Once again, the most prominent feature in each of these spectra is the trough in reflectance near 675 nm, which is confidently attributed to chlorophyll *a*. Small and variable troughs in reflectance occur at 600 nm, 800 nm, and 850 nm, and are expressed in some but not all samples (Figure 3.7A). Remarkably, the trough in reflectance that we attribute to chlorophyll is present even in the oldest samples (4-6 m), despite the loss of green coloration. Although this spectral feature becomes progressively more attenuated with sample distance from shore (*i.e.*, age), it can

Table 3.2. Correlation coefficients (r) between HPLC-measured pigment concentrations and reflectance indices selected from percent raw reflectance of samples in algal dilution series experiment, including both the pure inoculum and undiluted sediment matrix (n = 10). ** p <0.01; * p < 0.05; n.s.: not significant

Spectral index	Pigments		
	Chlorophyll *a* + isomers	Pheophorbide *a* + Pheophytin *a*	Chlorophyll *a* + derivatives
Reflectance trough area (between 650 and 750 nm)	0.90**	0.45*	0.99**
Amplitude of reflectance (between 675 and 750 nm)	0.74*	0.36*	0.69*
Reflectance ratio (675/750 nm)	0.21 n.s.	0.11 n.s.	0.28 n.s.
Amplitude of reflectance (between 650 and 675 nm)	0.15 n.s.	0.02 n.s.	0.14 n.s.
Reflectance ratio (650/675 nm)	0.08 n.s.	0.25 n.s.	0.08 n.s.

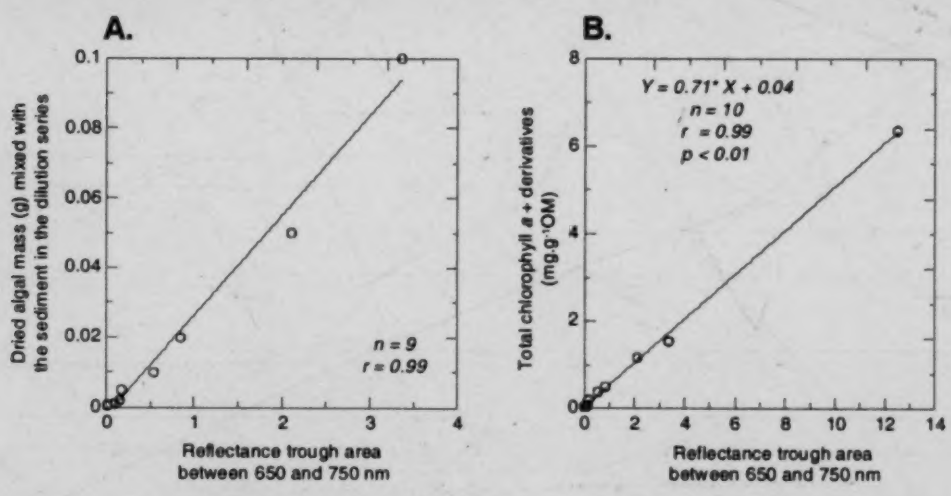

Figure 3.5. (A) Relationship between the reflectance trough area (between 650 and 750 nm) and the dry mass of algae mixed with sediment in the dilution series experiment. (B) Regression between the most highly predictive spectral index, reflectance trough area between 650 and 750 nm, and HPLC-measured total chlorophyll *a* and derivatives. In panel (B) the highest values is that of the pure algal inoculum, which was included in the inference model to constrain high chlorophyll concentrations.

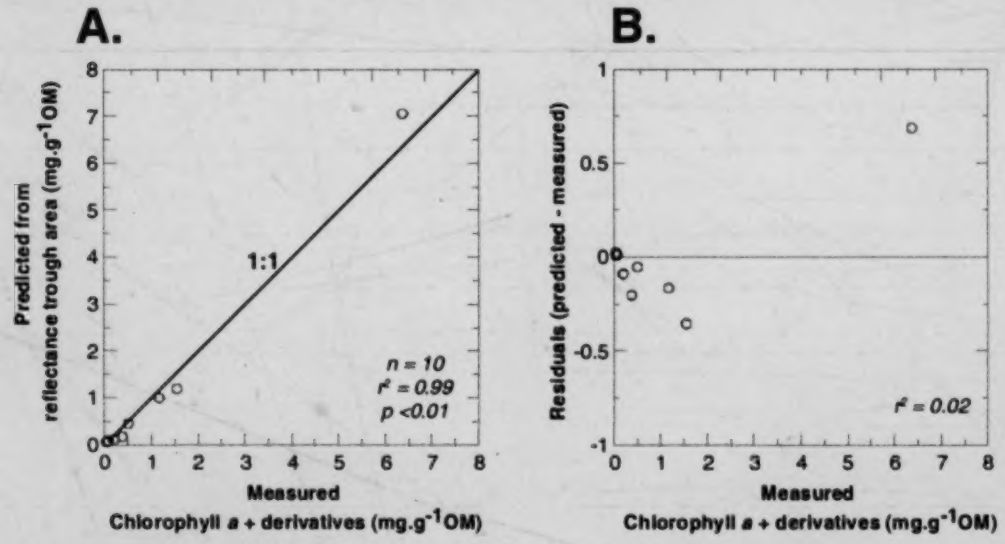

Figure 3.6. Comparison between measured (HPLC) and inferred total concentrations of chlorophyll *a* and derivatives from the reflectance trough area between 650 and 750 nm (A), obtained using the regression shown in Figure 3.5 B. The plot of residuals is shown in (B).

nonetheless be clearly identified in the oldest and most sun-bleached sample (Figure 3.7A). In fact, the reflectance trough area (650-750 nm) only decreases by 4% from the youngest to oldest sample, despite clearly intense photo-degradation (Figure 3.7 B), much more than is envisaged for typical lake sediments, once deposited.

Diagenetic processes and VNIR spectra: controlled redox conditions

Results from the aerobic and anaerobic incubations are presented in 28 day time steps (Figure 3.8). With time, VNIR reflectance spectra from both treatments decrease in overall amplitude. The trough in reflectance between 650 and 750 nm is, once again, well expressed at the beginning of the diagenesis simulations. By day 112, however, its amplitude has decreased in both oxic and anoxic treatments, but, as predicted, slightly more so in the oxic experiment. Interestingly, different patterns of progressive VNIR spectral evolution arise between the oxic and anoxic incubations (Figure 3.8 C). Up to 56 days, the greatest changes are expressed primarily between 500-600 nm, and again at wavelengths longer than 700 nm; changes in reflectance at 650 nm, near the maximum of chlorophyll absorption, do not vary between the treatments until later in the experiments, but even then the difference is relatively small (<1%; Figure 3.8 C).

The results of our simulations of both photo-degradation and redox conditions indicate that lake sediment VNIR spectra are potentially unstable during early diagenesis. On the other hand, the primary feature of interest for the purpose of reconstructing lake trophic history, the chlorophyll absorption band in the red portion of the spectrum, appears to be a relatively robust feature in all VNIR spectra investigated. One way of testing the implications of post-depositional spectral variability is by applying the simple

chlorophyll *a* inference model developed from the dilution series to a down-core sedimentary sequence, for which independent pigment HPLC determinations have been conducted.

Reconstructing lake trophic history with VNIR reflectance

Using down-core VNIR spectra, sedimentary concentrations of chlorophyll *a* and all derivative pheopigments were inferred from the sediments of Lac La Biche. These inferred values are directly comparable to HPLC measured concentrations (Figure 3.9 A). Though the VNIR inference model underestimates the surface samples, both techniques reveal the same trend: one of increased sediment chlorophyll concentrations as nutrient enrichment has progressed since the 1980s (Figure 3.9 A). Indeed, HPLC-measured and VNIR-inferred concentration of chlorophyll *a* and total derivatives are significantly correlated ($r^2 = 0.70$). Furthermore, these trends are independent of either those of sediment organic content, which is complacent in the upper 10 cm, or those of the chlorophyll *a* : pheophytin *a* ratio, which is highly erratic in the upper 15 cm. These results imply that the HPLC-measured and VNIR-inferred trends are not overprinted by diagenetic processes such as organic matter remineralization or chlorophyll degradation.

DISCUSSION

VNIR sensitivity to chlorophyll a in sediments

The key observation from the algal dilution experiment is that the depth of the trough in red reflectance (650-750 nm) deepens progressively with incremental additions of the algal inoculum. This trend is expressed without reversal (Figure 3.4 B). Given the

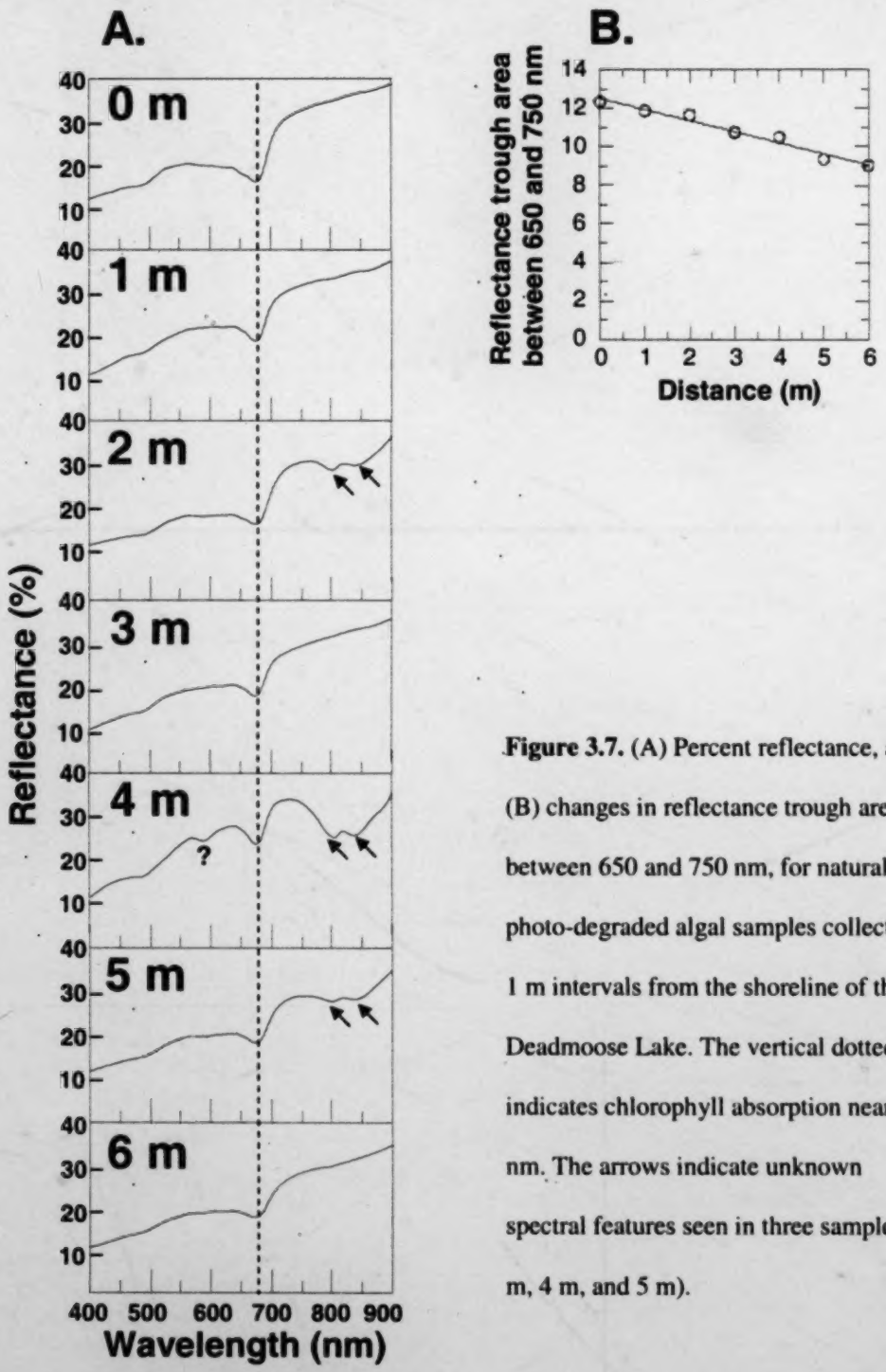

Figure 3.7. (A) Percent reflectance, and (B) changes in reflectance trough area between 650 and 750 nm, for naturally photo-degraded algal samples collected at 1 m intervals from the shoreline of the Deadmoose Lake. The vertical dotted line indicates chlorophyll absorption near 675 nm. The arrows indicate unknown spectral features seen in three samples (2 m, 4 m, and 5 m).

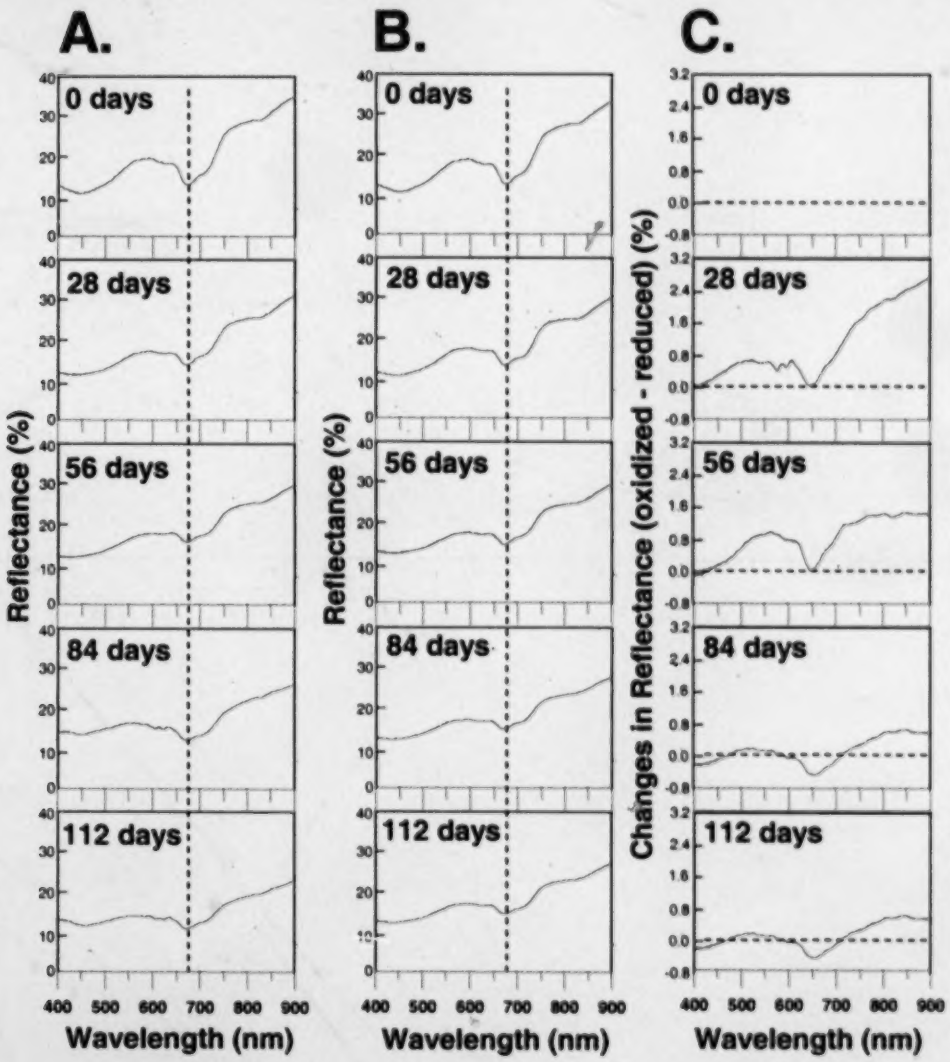

Figure 3.8. Percent VNIR reflectance of oxic (continuous aeration) (A) and anoxic (sealed) (B) samples showing the spectral changes near 675 nm. In panel (C) the difference between oxic and anoxic treatments (ΔR) are presented to illustrate time-dependent diagenesis of chlorophyll *a* and its impact on spectral VNIR reflectance.

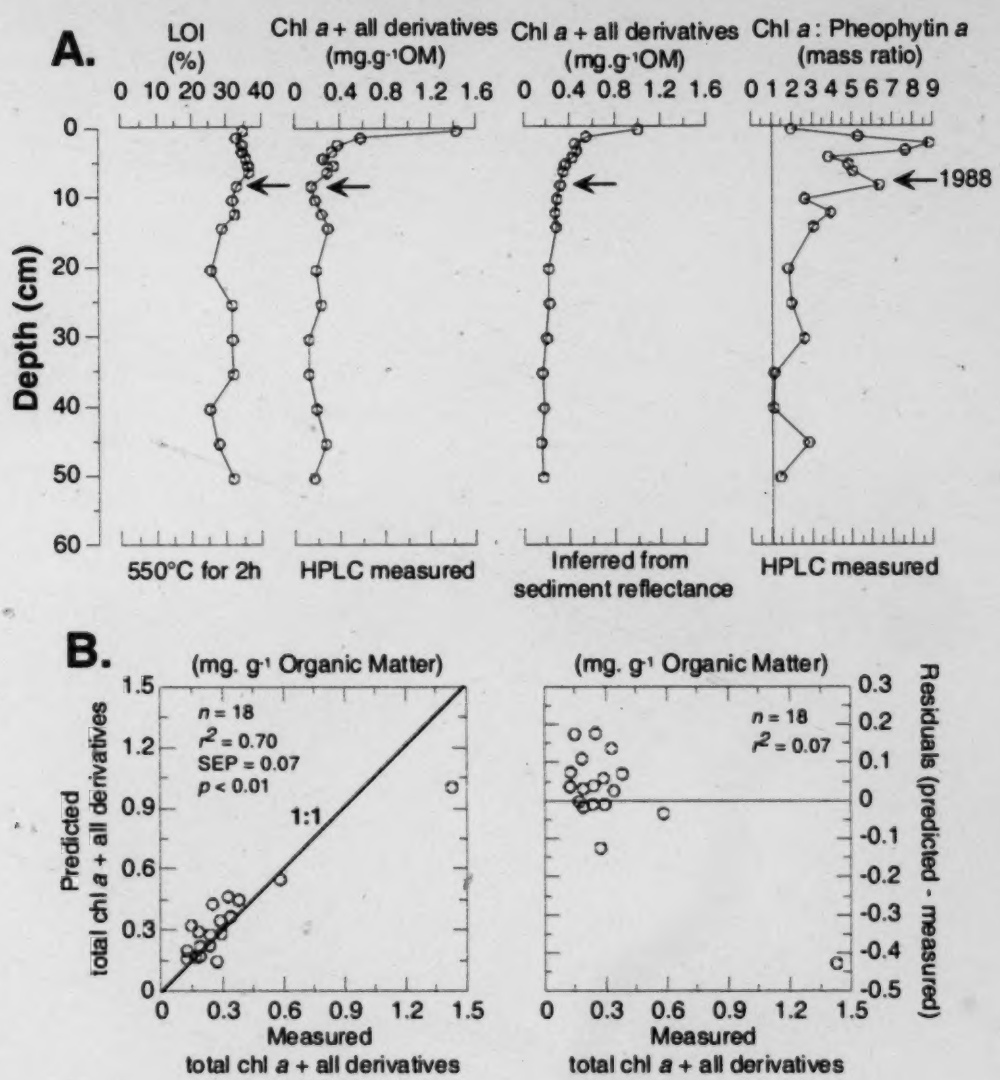

Figure 3.9. (A) Comparison of the HPLC-measured concentrations of total chlorophyll *a* and derivatives and the same quantity inferred from sediment reflectance in Lac La Biche sediments. LOI values and the chlorophyll *a* to pheophytin *a* ratio are shown to the right, and arrows indicate the year 1988 from ^{210}Pb dating. In (B) the 1:1 plot between HPLC-measured chlorophyll *a* and derivatives and VNIR-inferred values are shown, and residuals from this relationship.

pigmentary composition of the inoculum (Figure 3.3), coupled to the known sensitivity of this part of the electromagnetic spectrum to algal chlorophyll in water (Rundquist et al. 1996; Schalles et al. 1998), we confidently ascribe the shape of the trough in sediment reflectance centered at 675 nm to the concentration of chlorophyll in the sample. It is thus possible to exploit this spectral feature as a predictor of sedimentary chlorophyll.

The amplitude of VNIR reflectance changes directionally with the amended dry mass of algae added to the sediment matrix, which in turn relates to total concentrations of chlorophyll *a* and derivative pheopigments (Figures 3.4 C and D). The minimum of visible reflectance (400-600 nm) with higher pigment concentration is apparently an outcome of strong absorption by organic matter including chlorophylls, suggesting that absorption is much more effective than scattering in most of the visible portion of the spectrum (Gitelson et al. 1999). Increased glycolipids along with chlorophyll *a* concentrations lead to increased scattering, which might be also related to other fossil constituents in sediment samples and hence higher reflectance in the red-near infrared region (675 to 900 nm). The VNIR spectral characteristics of sediment are thus perfectly compatible with those of living algal biomass in water (Schalles et al. 1998; Gitelson et al. 1999), providing an essential validation of the observations reported here.

Preservation of chlorophyll VNIR spectral signatures in lake sediments

The progressive dampening of the chlorophyll reflectance trough from the least (0 m) to the most highly (6 m) sun-bleached littoral samples from Deadmoose Lake confers a certain degree of VNIR sensitivity towards intense photo-degradation. However, these degrees of bleaching are well beyond what may potentially occur *in situ* at the bottom of

lakes, implying that only a small fraction of autochthonous production (*e.g.* dead phytoplankton that remains suspended for prolonged periods, reworked littoral sediment, *etc.*) will be degraded to this extent. Furthermore, even the most extreme sun-bleaching only impacts VNIR inferences of chlorophyll *a* and derivatives by <0.05 mg g^{-1}OM (Figure 3.10), so that, overall, the influence of this process are unlikely to mask trend mediated directly by lake primary production.

Differences between the VNIR spectra of samples incubated under oxic *versus* anoxic conditions indicate that oxidative processes strongly influence reflectance initially (28 and 56 days), but relatively little thereafter (Figure 3.8 C). This result is consistent with other experiments in which most early diagenetic transformations occur very rapidly from the outset, especially under oxic conditions (Lehmann et al. 2002). In the present experiments, however, the trough near 675 nm remained the dominant VNIR spectral feature throughout the experiments, despite slight dampening of overall reflectance observed in both oxic and anoxic trials. When these trends are converted to VNIR-inferred concentrations of chlorophyll *a* and derivatives, using the model developed from the dilution series, the apparent losses of these compounds are four-fold greater over 112 days under continuous aeration (0.08 mg g^{-1}OM) relative to anoxic conditions (0.02 mg g^{-1}OM). These results indicate that apparent amounts of chlorophyll accounted for by diagenesis, even under strong and continuous oxidation, are relatively small by comparison to average concentrations in typical lake sediments (compare: Figures 3.9 A and 3.10).

Finally, because primary chlorophyll *a* and its degradation products (pheophytin *a* and pheophorbide *a*) absorb in almost identical regions of the electromagnetic spectrum

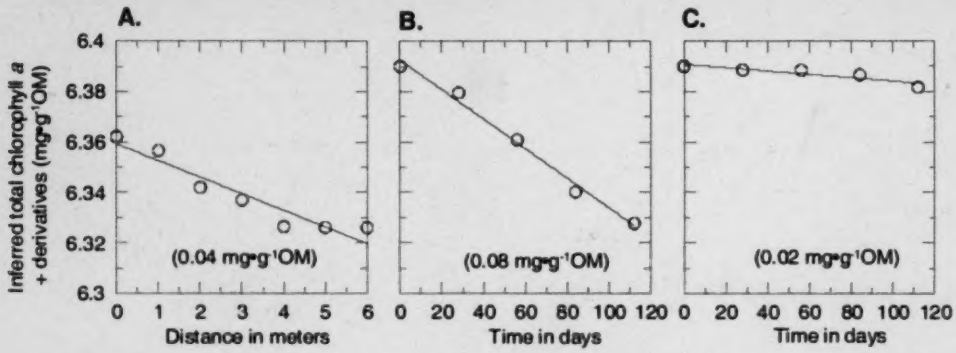

Figure 3.10. Changes in VNIR-inferred concentrations of total chlorophyll *a* and derivatives among the three analyzed diagenetic pathways: photo-degradation (A), oxidation (B), and reduction (C). All inferences are based on the VNIR reflectance trough area index and the model developed from the algal dilution series experiment. Values in parenthesis indicate apparent losses of (inferred) sediment chlorophyll due to diagenesis.

(Méléder et al. 2003), 'steady-state' progressive transformation of primary photosynthate to pheopigments is unlikely to strongly influence VNIR inferences, which is viewed as a

major strength of the technique. Significant correlations between several spectral indices and both pigment classes verify this to be the case (Table 3.2; see also: Das et al. 2004, Chapter 2). Thus, sediment VNIR spectral features should potentially prove fruitful in reconstructing lake trophic regimes on a variety of time-scales. One example of this application is assessed in the following section.

Reconstructing paleoproduction from sediment VNIR reflectance

The recent cultural eutrophication of Lac La Biche is associated with increased nutrient loading from agricultural and municipal sources, coupled to drought-induced lake-level lowering and attendant increases in water residence times (Mitchell and Prepas 1990). Both HPLC-measured and VNIR-inferred concentrations of total chlorophyll *a* and derivatives from sediments chronicle these changes faithfully, showing enrichment after 1988 (Figure 3.9 A). Fluctuating values of chlorophyll *a* to pheophytin *a* ratio are observed in the uppermost eight samples (0-8 cm), indicating highly unstable condition, whereas the accompanying trend in summed chlorophyll *a* and derivatives, both measured and inferred, increase progressively towards the surface. The parallel behaviour observed between measured and inferred total chlorophyll *a* stratigraphies is reflected not only by the shape of their respective trends, but also by their absolute concentrations, as shown by a close clustering to the 1:1 line and reduced scatter among residuals, with the exception of the surface sample which is underestimated by VNIR (Figure 3.9 B). These results confirm the appropriateness of VNIR for rapid and non-destructive semi-quantitative determinations of chlorophyll *a* and its derivatives from lake sediments. For applications in lake management that require baseline historical lake trophic status to be

determined rapidly and cost-effectively, the VNIR techniques reported here appear well-suited.

CONCLUSIONS

A robust red absorption feature attributed to total chlorophyll *a* and its derivatives is consistently identified in lake sediment VNIR spectra. Furthermore, the expression of this feature in sediment samples originating from a wide variety of lake types (saline, eutrophic, and oligotrophic, see also: Das et al. 2004, Chapter 2) implies that this signature is pervasive, which is a logical consequence of the ubiquity of chlorophyll *a* as a primary photosynthates shared among aquatic autotrophs, irrespective of their ecologies. This suggests that VNIR-based techniques are likely to remain sensitive for all lake types where chlorophyll *a* is preserved in sediments.

From the experiments reported here, diagenesis proceeds as expected for both photo-degradation and redox-mediated processes, by progressively muting the sharpness of VNIR spectra in the red and thus producing slight attenuations in the inferred concentrations of total chlorophyll *a* and its derivatives. The major constraint of the technique is its inability to separate primary chlorophylls from chlorophyll derivatives, or resolve trace pigments such as carotenoids. On the other hand, reflectance spectroscopy is nonetheless a powerful tool for rapid and non-destructive quantification of total sedimentary chlorophyll *a*. The results presented here demonstrate that VNIR reflectance spectroscopy can be applied to the reconstruction of historical changes in lake trophic, a major objective in many paleolimnological investigations.

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CHAPTER FOUR: General Conclusions

Proper management of lake eutrophication requires long-term data in order to assess when, and by how much, a lake has eutrophied. Paleolimnological techniques can provide these missing data sets. In this thesis, it has been established that visible-near-infrared (VNIR) reflectance spectroscopy is a potentially powerful tool for reconstructing paleoproductivity from recent (<200 years old) lake sediments. The exploratory, experimental, and applied results of this study demonstrate that VNIR spectral properties, specifically the red-near-infrared region of the electromagnetic spectrum (~600-750 nm), are highly sensitive to the total concentration of chlorophyll *a* and its derivatives in sediments from a variety of lake types. These findings are thus perfectly compatible with prior work on the reflectance characteristics of living algal cells (Schalles et al., 1998, Méléder et al., 2003).

Chapter 2 demonstrates that chlorophyll *a*, as detected from reflectance spectroscopy, showed a similar response to non-point-source nutrient enrichment as that borne out by independent proxies (i.e., diatom assemblages, Wolfe et al., 2003). The implication of this initial finding is that VNIR reflectance-inferred chlorophyll *a* is potentially viable rapid and non-destructive (and hence cost-effective) method for reconstructing lake paleoproductivity. However, the possibility of diagenetic overprinting in VNIR spectra, which would confound the straight-forward interpretations suggested by Chapter 2, remained to be addressed at a sufficient level of detail.

In Chapter 3, it is demonstrated that both photo-degradation and redox state play only secondary roles in shaping sediment VNIR spectra. This is because both primary chlorophyll *a* and its degradation products share absorption in the critical region used to

infer primary photosynthate: the red-nearest infrared (600-750 nm). Thus, the reflectance trough centered near 675 nm is dependent on the summed concentrations of chlorophyll *a* and its derivatives, or the complete chlorophyll *a* 'family', a result that largely obviates the influence of diagenetic processes.

Future applications

The results of this thesis are limited to relatively short time scales (< 200 years). Clearly, it is relevant to extend these techniques to older lake sediment sequences, all the more since lake production can be linked to climate through several mechanisms (Flanagan et al., 2003). This potential appears supported by initial results of VNIR analyses of sediments spanning the Holocene (Rosén et al., 2000), although chlorophyll was not targeted by this study. While reconstructing recent trophic histories has implications with respect to management of lake eutrophication, still a serious threat to aquatic ecosystems (Schindler, 2000), the linkage to climate change is perhaps more tantalizing still. This is because climate influences lake primary production through both direct mechanisms such as prolongation of the growing season and modification of lake thermal structure, as well as indirect mechanisms such as the regulation of catchment nutrient fluxes (Schindler et al., 1990, Hobbie et al., 1999). Some of these ideas may be tested, for example, by examining lake paleoproductivity regimes using VNIR methods, for the warmer-than-present climate states of the early Holocene. Clearly this approach has the potential to examine the response of lake systems, at different timescales, to climate changes forced by both natural and anthropogenic factors.

But chlorophyll is certainly not the only discrete sediment constituent that can be explored using VNIR reflectance spectroscopy. For example, charcoal is common in lake sediments and widely used to reconstruct fire histories, which, of course, are also linked to climate variability (Whitlock and Larsen, 2001). Once again, the microscopic techniques currently used to quantify charcoal are labor-intensive and time-consuming. Reflectance spectroscopy may potentially be sensitive to trace amounts of carbon in sediment samples, because charcoal is highly opaque. Pilot studies on the VNIR spectra of charcoal and charcoal-bearing lake sediments have been initiated.

Another potential application of reflectance spectroscopy involves longer wavelengths, particularly those that characterize thermal infrared emissions (5 - 25 μm). These wavelengths are more sensitive to inorganic as opposed to organic molecules, and have proven useful with respect especially to silicates (Hamilton, 2000). Biogenic silica ($n H_2O \cdot SiO_2$) is an amorphous biomineral that is produced primarily by siliceous algae (diatoms and chrysophytes), with minor contributions from protozoa (rhizopod thecal plates), sponges (spicules), and grasses (phytoliths). Biogenic silica in lake sediments is a powerful proxy for understanding the lacustrine silica cycle, which is linked to both weathering processes and diatom production, both of which relate to climate history. The typical wet-chemical methods for biogenic silica determination are tedious and time-consuming (Conley and Schelske, 2001). Given that the thermal infrared spectrum of biogenic silica has a single broad absorption band between 7.7 μm and 10.5 μm due to Si-O stretching, and additional absorption at 21 μm (Si-O bending) (Michalski et al., 2003), it seems possible that biogenic silica may be inferred from lake sediments in much

the same way as chlorophyll, only exploiting significantly longer wavelengths. Pilot studies in this area are also progressing, with promising initial results.

In summary, the discipline of paleolimnology may clearly benefit from careful applications of sediment VNIR spectral properties, providing yet another tool for reconstructing the rate and tempo of environmental change that is archived in lake sediments. The power of VNIR reflectance spectroscopy lies in the rapidity and reproducibility of analyses, which are augmented greatly by the fact that measurement is non-destructive. These qualities imply, as we understand more and more about the significance of lake sediment VNIR spectra, that meaningful analyses of lake cores at unprecedented stratigraphic (~temporal) resolution may become a reality. Similarly, the analysis of multiple cores from a single lake, which is currently seldom achieved due to time constraints, also becomes more realistic, and is clearly relevant in assessing the fidelity of inferences based on single cores. This thesis has shown several instances where VNIR reflectance spectroscopy can be used as a viable alternative to wet-chemical analysis of lake sediment chlorophyll *a*. However, it is emphasized that VNIR is a method that complements, rather than supplants, traditional approaches that remain much needed for both adequate calibration of spectra l reflectance data, as well as quantification of trace compounds that remain 'invisible' in VNIR reflectance spectroscopy. However, the integration of both methods in studies such as this one may expand the ability to infer changing regimes of lake production at several spatial and temporal scales.

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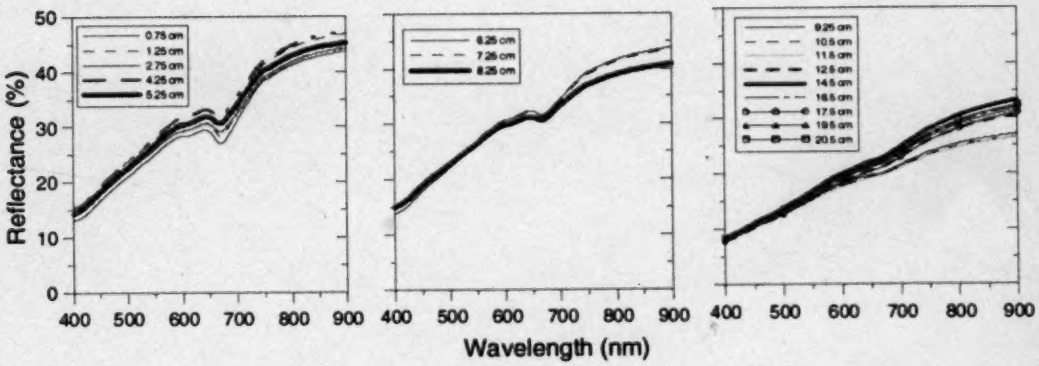
APPENDICES

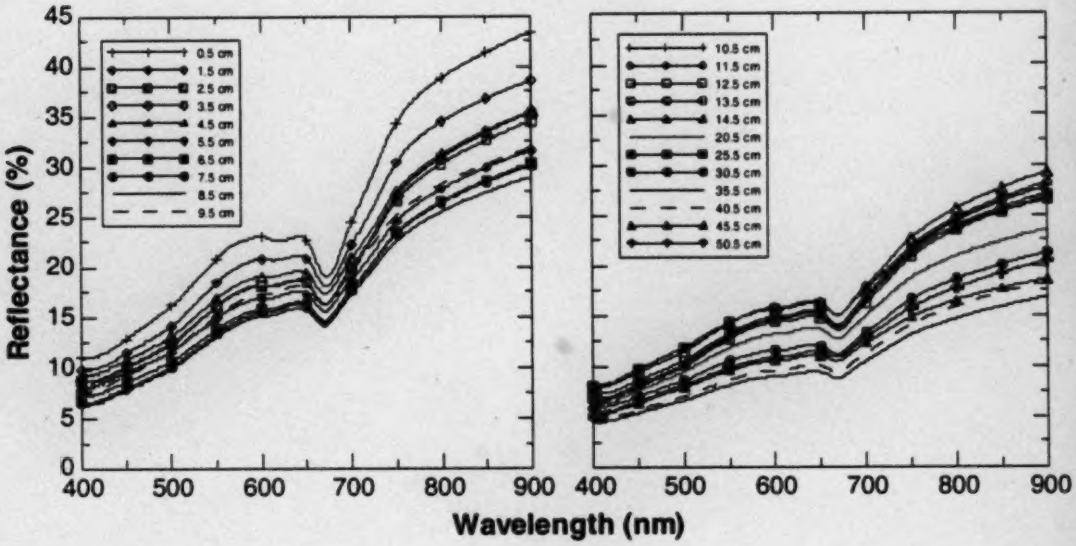
APPENDIX 1: Sediment reflectance spectra of an alpine lake at different depths

(Pristine Lake, Mount Zirkel Wilderness Area, USA)



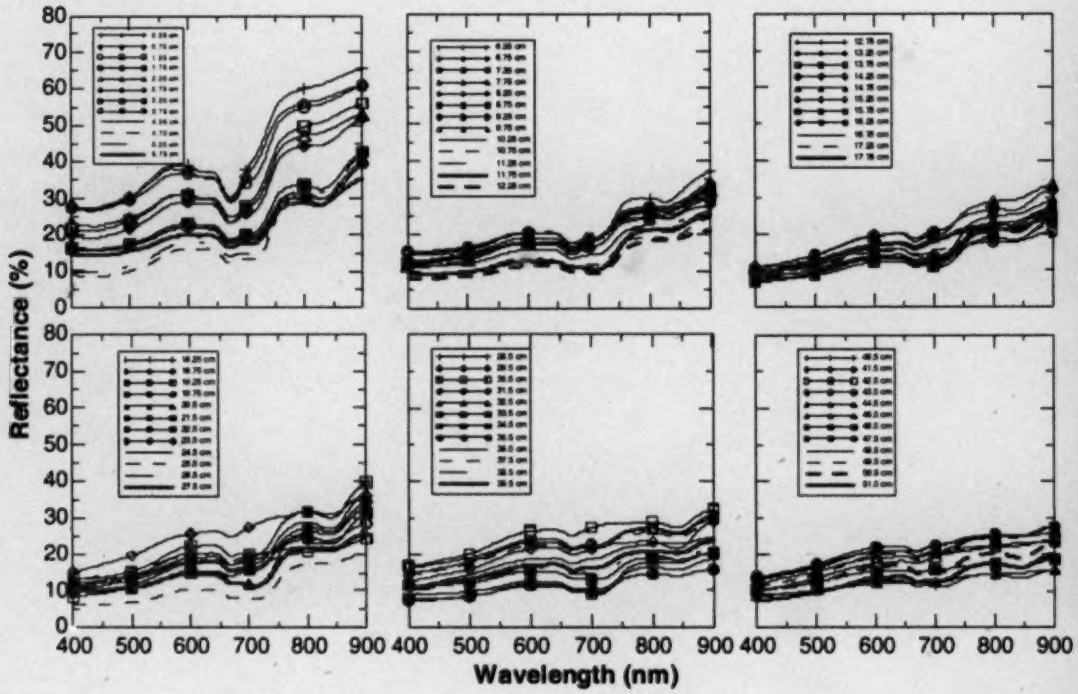
APPENDIX 2: Sediment reflectance spectra of a boreal lake at different depths

(Lac La Biche, Alberta)



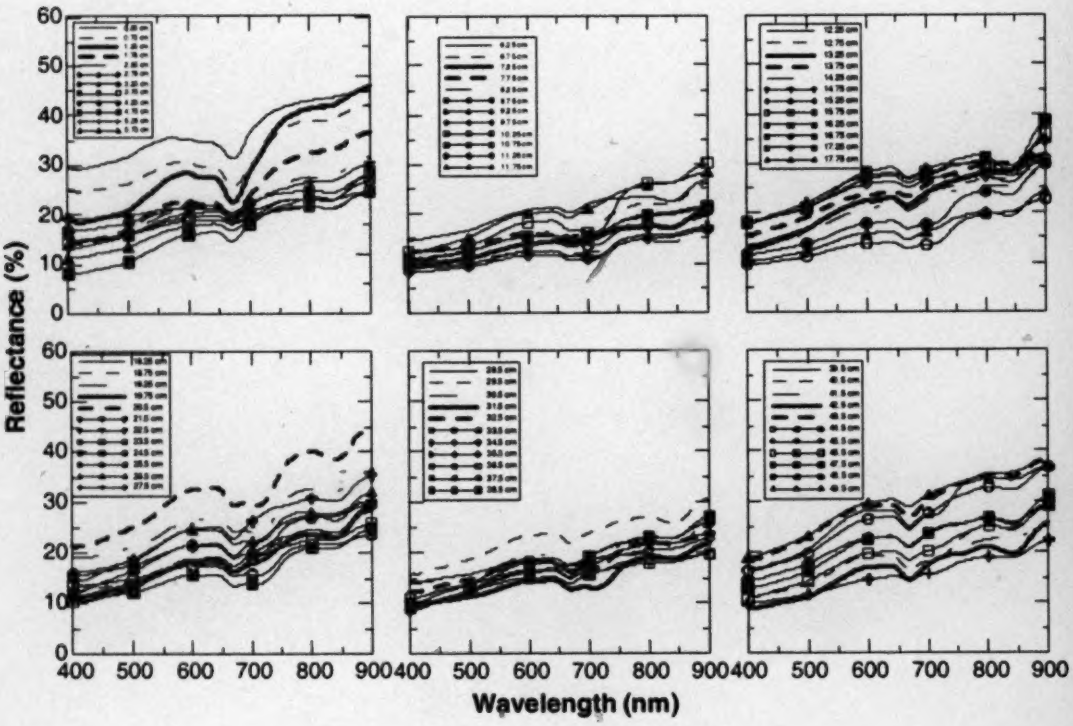
APPENDIX 4: Sediment reflectance spectra of a saline lake at different depths

(Waldsea Lake, Humboldt, Saskatchewan, Canada)



APPENDIX 5: Sediment reflectance spectra of a saline lake at different depths

(Deadmoose Lake, Humboldt, Saskatchewan, Canada)



APPENDIX 6: Information on archived samples (dilution series)

Sample Description

Pre-industrial lake sediments from Lake Louise (22.5 cm depth, approximately 200 years old; Wolfe et al., 2003) were used as the matrix for the algal dilution series. Lake Louise is located in Rocky Mountain National Park (RMNP), Colorado, USA. The algal inoculum used for the dilution series experiment was collected from the bottom of experimental fish-rearing aquaria. The inoculum comprised mixed colonial, coccoid, and filamentous cyanophytes, dominated by *Oscillatoria* and *Microcystis* spp. Bulk algal samples were freeze-dried, ground, and mixed with the sediment at the following sediment:algae mass ratios: 5:1, 10:1, 25:1, 50:1, 100:1, 250:1, 500:1, 750:1, and 1000:1. Chlorophyll *a* concentrations, as well as those of associated pheopigments, were also determined on these samples by HPLC, to allow the development of a simple calibration between VNIR spectra and absolute sediment pigment concentrations.

Sample ID

Sample #1: Sediment matrix

Sample #2: sediment:algae mass ratio 5:1

Sample #3: sediment:algae mass ratio 10:1

Sample #4: sediment:algae mass ratio 25:1

Sample #5: sediment:algae mass ratio 50:1

Sample #6: sediment:algae mass ratio 100:1

Sample #7: sediment:algae mass ratio 250:1

Sample #8: sediment:algae mass ratio 500:1

Sample #9: sediment:algae mass ratio 750:1

Sample #10: sediment:algae mass ratio 1000:1

Compact Disk contents

VNIR reflectance spectra of these samples AND the corresponding pigment concentrations measured by high pressure liquid chromatography (HPLC) in EXCEL format.

Professional organizations and membership

- Geological Association of Canada

Publications and presentations

Articles published or submitted in referred journals

- **Das, B.**, Wolfe, A. P., Vinebrooke, R. D., and Rivard, B. Visible-near-infrared (VNIR) spectral characterization of chlorophyll in lake sediments (manuscript destined for the *Journal of Paleolimnology*, in final preparation).

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Conference presentations

- **Das, B.,** A. P. Wolfe, and R. Vinebrooke. Spectral inferences of lake sediment chlorophyll: a potential tool for reconstructing paleoproductivity. Presented in the 49th Annual Meeting "Lake to Lake" of the Geological Association as well as the Mineralogical Association of Canada, St. Catharines, ON, 12-14 May 2004.

- **Das, B.** and A. P. Wolfe. Application of reflectance spectroscopy in analysis of lake sediment chlorophyll *a* from alpine, arctic, eutrophic and salty lakes. Abstract accepted for presentation in the Geo-Environment 2004 conference, 5-7 July 2004, Segovia, Spain.

- Participated in the focus group discussion and validation (university level) on National Occupation Standard (NOS) for the environmental practitioners organized by the Canadian Council for the Human Resources in the Environmental Industry (CCHREI), Calgary, Alberta, Canada, 25th November 2003.

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